

PLASMA LIPOPROTEIN ALTERATIONS IN THYROID DYSFUNCTION

Roles of lipoprotein lipase,
hepatic lipase and LCAT

Stig Valdemarsson



Lund 1983

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hepatic lipase and LCAT**

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To

Lena, Martin and Johan

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- I. Relations between thyroid function, hepatic and lipoprotein lipase activities, and plasma lipoprotein concentrations.
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Acta Endocrinologica. In press.
- II. Reversal of decreased hepatic lipase and lipoprotein lipase activities after treatment of hypothyroidism.
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- III. Changes in plasma lipoprotein concentrations and composition during replacement therapy in hypothyroid patients: Relation-ship to alterations in lipolytic enzymes and LCAT.
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Stig Valdemarsson, Pavo Hedner and Peter Nilsson-Ehle.
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BACKGROUND

Changes in plasma lipid concentrations are well known metabolic consequences of thyroid dysfunction. The alterations are most prominent in hypothyroidism which is typically associated with pronounced hypercholesterolemia and frequently also with moderate hypertriglyceridemia. Hyperthyroidism, on the other hand, is often accompanied by decreased levels of serum cholesterol. Although the pathophysiological mechanisms for these lipoprotein abnormalities are not clarified, it is well known that these changes are completely reversible. Since thyroid disease is rarely associated with secondary alterations in other organs involved in plasma lipoprotein metabolism, patients with thyroid dysfunction provide an unusually clean model for dynamic studies on the regulation of lipoprotein metabolism in man. The present investigations focus on the relationships between thyroid hormones and key enzymes in lipoprotein metabolism, and on the impact of enzyme alterations on the plasma lipoprotein profile. Besides improving our understanding of the pathogenesis of the dyslipoproteinemias in thyroid dysfunction per se, such information may be of importance also for the interpretation of lipoprotein alterations in general. Since lipoprotein abnormalities rank among the major risk factors for coronary heart disease, such knowledge may in turn be of value for our understanding and clinical approach to atherosclerotic disease.

Lipoprotein metabolism and the roles of lipoprotein lipase, hepatic lipase and lecithin:cholesterol acyltransferase

Plasma lipids - cholesterol, triglycerides and phospholipids - are transported in the blood stream complexed with specific proteins - the apolipoproteins - in spherical particles, the lipoproteins (Fig. 1). Triglyceride and cholesteryl ester constitute the core of these particles, and are covered by a surface layer of amphipathic components, i.e. phospholipids, unesterified cholesterol and apolipoproteins. Three major lipoprotein classes can be separated, according to their

Table 1

Physiochemical properties, half life and composition of plasma lipoproteins in healthy subjects

	Half life	Electro-phoretic mobility	Particle diameter	Density	Approximative relative (w/w) composition				Major apo-lipo-protein
			nm	kg/l	TG	Chol	Esterified chol	Phospholipid	
Chylomicrons	min.	origin	> 75	< 0.95	84	2	5	7	2 apo B
VLDL	hours	pre	25-75	0.95-1.006	55	7	12	18	8 apo B
IDL	min. ?	pre-pre β	22-24	1.006-1.019	-	-	-	-	-
LDL	5-9 days	β	20-23	1.019-1.063	6	8	42	22	22 apo B
HDL-2	5 days	α	6-14	1.063-1.125	5	5	17	31	42 apo A
HDL-3	5 days	α	4-10	1.125-1.210	4	4	13	24	55 apo A

different densities, from plasma of a fasting subject: high density lipoproteins (HDL), with a large proportion of protein; low density lipoproteins (LDL), the main carrier of cholesteryl ester in plasma; and very low density lipoproteins (VLDL), which are dominated by triglyceride. Another class of triglyceride-rich lipoproteins, the chylomicrons, appear in postprandial plasma, transporting fat from the intestine to the tissues. Some properties and the composition of the lipoproteins are given in Table 1.

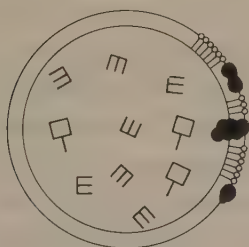


Fig.1. Schematic outline of the structure of plasma lipoproteins. The hydrophobic core components, i.e. triglyceride (E) and cholesteryl ester () are covered by a surface film consisting of amphipathic components, i.e. apolipoproteins (), phospholipid and unesterified cholesterol ().

Current views on lipoprotein metabolism in plasma are summarized in Fig. 2 (Nilsson-Ehle et al. 1980, Brown et al. 1981, Nikkilä et al. 1982, Patsch 1982, Eisenberg et al. 1982). VLDL particles are synthesized in the liver and secreted into the circulation. During their passage through the capillaries in various organs, these particles are acted upon by lipoprotein lipase (LPL). This enzyme is synthesized by parenchymal cells in e.g. adipose tissue, heart and skeletal muscle, and is

secreted from these cells and transported to the endothelial cells of the capillary vessels where it has its site of action. LPL catalyzes the degradation of triglyceride in the core of VLDL and chylomicrons. The lipolytic products are assimilated into the tissues for oxidation or storage. Once initiated, the lipolytic process normally proceeds quite rapidly, leading to the formation of LDL particles which are almost completely depleted of triglyceride. However, impairment of the lipolytic process may, in certain pathological conditions, result in the accumulation of partially degraded particles of intermediate density (IDL). In normal plasma such IDL particles can barely be detected. The LDL particles, which are the main carrier of cholesterol through plasma, deliver cholesterol to peripheral cells by the uptake of the LDL particle in the cell via specific LDL receptors (Goldstein & Brown 1977, Brown et al. 1981). The uptake of LDL particles may also occur, at least at high plasma cholesterol levels, via non-adsorptive pinocytosis (the scavenger pathway), independent of the LDL receptor system.

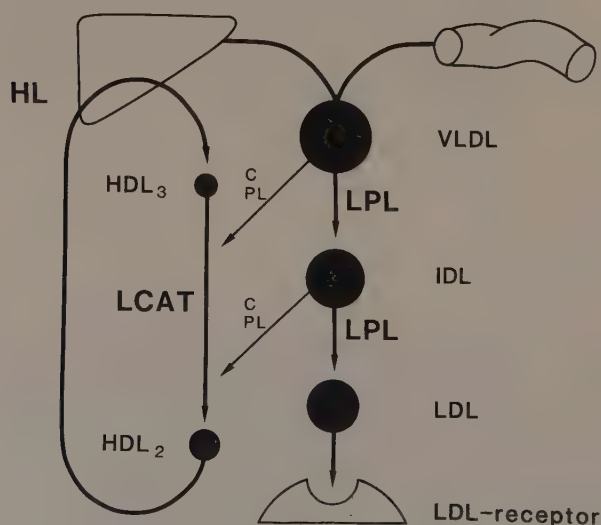


Fig. 2. Hypothetical model for the intravascular lipoprotein metabolism. PL, phospholipid; C, unesterified cholesterol.

In parallel with the reduction of the core of the triglyceride-rich particles, there will be an excess of surface components, i.e. of unesterified cholesterol, phospholipids and apolipoproteins. These components are transferred to another lipoprotein system, the high density lipoproteins (HDL). These particles are originally synthesized by the liver and carry an enzyme system, lecithin: cholesterol acyltransferase (LCAT), which catalyzes the further processing of the surface components (Norum et al. 1982). Cholesterol and phospholipids are transesterified, resulting in the formation of lysolecithin (which leaves the lipoproteins and binds to albumin) and cholesteryl ester. While some of the cholesteryl ester formed is transferred back to VLDL and LDL particles by specific cholesteryl ester transfer proteins (Marcel et al. 1980, Fielding & Fielding 1981), some is retained and incorporated into the core of the HDL particle. This process leads to the transformation of smaller, denser HDL particles (HDL₃) to larger, less dense HDL₂ particles (Patsch et al. 1978). There are data indicating that the transfer of components from the triglyceride-rich lipoproteins to the HDL fraction may differ qualitatively for VLDL and chylomicrons. The latter particles carry all components necessary to form an HDL particle, and it has been suggested that surface components are dissociated from the shrinking chylomicron in particulate form, rather than by molecular net transfer to preformed HDL particles. It is possible, therefore, that HDL may partly be indirectly derived from the intestine (Tall & Small 1978).

Besides acting as an acceptor for cholesterol and phospholipids from triglyceride-rich lipoproteins, HDL has also been suggested to take part in a reverse transport of cholesterol from peripheral cells to the liver (Glomset 1972). The liver is the only organ that can excrete cholesterol from the body. It has recently been suggested (Nikkilä et al. 1982) that HDL may act as a shuttle, where cholesteryl ester loaded HDL₂ particles convey cholesterol and phospholipids to the liver. After partial degradation the HDL particle returns to the circulation, as HDL₃, again prepared to pick up more cholesterol and phospholipid. The concept of HDL as an important factor for

the maintenance of optimal cholesterol concentrations in the tissues is the theoretical basis for its "antiatherogenic properties", as originally indicated by epidemiological studies (Miller & Miller 1975).

There are, however, observations that some extrahepatic cells are also able to utilize HDL cholesterol. Animal experiments have shown that adrenal cortex and ovarian tissue (Brown et al. 1979) can take up HDL cholesterol from plasma. Therefore, HDL may have a role both for the elimination of cholesterol from the body and for the distribution of cholesterol between different tissues.

The enzyme hepatic lipase (HL) has been suggested to play an important role in the metabolism of HDL particles during their passage through the liver. HL is synthesized by the hepatocytes, but has its site of action at the endothelium of the hepatic sinusoids (Kuusi et al. 1979 b). The exact physiological function of HL remains to be settled, but there is increasing evidence that it acts as a phospholipase (Ehnholm et al. 1975 a, van Tol et al. 1980). In vitro studies have shown that HDL₂ phospholipid is the preferred substrate for HL (Shirai et al. 1981). As suggested by Nikkilä et al. (1982), the physiological role of HL would be to degrade phospholipids of HDL₂ particles, which would also facilitate cholesterol assimilation by the liver cells for further degradation and excretion. The modified HDL particles would then re-enter the circulation as HDL₃ particles. By catalyzing this process, HL could regulate the hepatic elimination of cholesterol from triglyceride-rich lipoproteins and from peripheral cells via the HDL system.

This hypothetical model for the intravascular metabolism of plasma lipoproteins is based on a variety of clinical and experimental observations; still certain aspects may be controversial. However, its value and validity is supported by the fact that predictions from the scheme frequently conform with reality. For example, differences (or changes) in LPL activity would lead to an inverse relationship between VLDL and HDL concentrations; such an inverse relation-

ship has been repeatedly demonstrated in cross-sectional as well as in longitudinal investigations (Nichols 1967, Fredrickson et al. 1967, Wilson & Lees 1972). Plasma HDL concentrations would be influenced by both LPL and HL activities, promoting the input into and the elimination of cholesterol from the HDL system, respectively. Indeed, there are data from clinical studies supporting this view, indicating that these enzymes may be of major importance for the determination of plasma HDL concentrations (Belfrage et al. 1977, Nikkilä et al. 1978, Kuusi et al. 1980, Agardh et al. 1983).

Lipoprotein metabolism in thyroid dysfunction

A relationship between serum lipid concentrations and basal metabolic rate was recognized already in 1922 (Epstein & Lande 1922). Elevated levels of serum cholesterol in hypothyroidism was reported by Mason et al. (1930). This observation was confirmed about 20 years later (Peters & Man 1950, Malmros & Swahn 1953) and it became evident that hypercholesterolemia was one of the most consistent metabolic concomitants of primary forms of thyroid hypofunction. In contrast, it is frequently stated that the increase in serum cholesterol levels is smaller or even absent in hypothyroidism secondary to pituitary failure (Bastenie et al. 1972, Utiger 1979, Dillon 1980, Ingbar & Woeber 1981, Christy 1982). Studies on the different lipoprotein classes in patients with thyroid disease revealed characteristic changes especially in the low density lipoproteins. In 1965, Walton et al. (1965 a) demonstrated that patients with primary hypothyroidism had increased serum levels of cholesterol and phospholipids, while the opposite pattern was found in hyperthyroid patients. These changes could be ascribed to reversible changes in the metabolism and turn over of low density lipoproteins: the catabolic rate of LDL was decreased in hypothyroidism, while it was increased in hyperthyroidism (Walton et al. 1965 b). In 1968, Miettinen reported that the decrease in serum cholesterol levels during thyroxine replacement in myxedematous patients was associated with a marked increase in fecal excretion of neutral steroids derived from circulating cholesterol.

Serum triglyceride levels are in general reported to be elevated in hypothyroidism (Nikkilä & Kekki 1972, Tulloch et al. 1973, Rössner & Rosenqvist 1974), while only moderate or no changes are seen in hyperthyroidism (Nikkilä & Kekki 1972, Tulloch et al. 1973, Agdeppa et al. 1979). Nikkilä & Kekki (1972) found a marked reduction of the fractional removal of triglyceride in hypothyroidism. In hyperthyroidism, on the other hand, removal of plasma triglyceride was accelerated, but simultaneously the production rate of triglyceride was increased, leading to essentially normal plasma triglyceride concentrations. Similar data on triglyceride metabolism in hypo- and hyperthyroidism have recently been reported by Abrams et al. (1981); in addition these authors pointed out that the disturbance in triglyceride metabolism in hypothyroid patients could to some extent be influenced by the presence of obesity in these patients. The defect elimination of triglyceride from the circulation in hypothyroid patients is also illustrated by an impaired elimination of exogenous triglyceride (Nikkilä & Kekki 1972, Rössner & Rosenqvist 1974).

Further studies on the lipoprotein pattern in thyroid hypofunction corroborated and extended the observations that the changes occur mainly in the LDL fraction (Wahlqvist et al. 1977, Ballantyne et al. 1979, Agdeppa et al. 1979). More delicate separations of the lipoproteins in these patients revealed accumulation of particles in fractions between VLDL and LDL (Ballantyne et al. 1979), i.e. in subclasses corresponding to the IDL fraction.

While the changes in the VLDL - LDL system are marked and consistent, data on HDL concentrations in thyroid disease are more difficult to evaluate. In general there seems to be only minor changes in total HDL cholesterol levels in hypothyroidism (Rössner & Rosenqvist 1974, Agdeppa et al. 1979, Ballantyne et al. 1979, Scottolini et al. 1980), while moderately decreased HDL cholesterol levels is a more consistent finding in hyperthyroidism (Agdeppa et al. 1979, Scottolini et al. 1980).

In a review from 1973, Evered et al. point out that hypothyroidism should be regarded as a graded phenomenon. In their study significantly increased levels of serum cholesterol appeared only in patients with overt hypothyroidism. Patients with mild or subclinical forms of hypothyroidism have been discussed to have an increased risk for coronary heart disease (Vanhaelst & Bastenie 1979). So far, however, no significant changes have been observed neither in serum cholesterol nor in serum triglyceride levels in subclinical hypothyroidism, at least as long as serum thyrotropin (S-TSH) levels do not exceed 40 mU/l (Nilsson et al 1976, Kutty et al. 1978, Lithell et al. 1981).

While the lipoprotein patterns in patients with thyroid disease are fairly well characterized, the pathophysiological mechanisms behind these disturbances are still unclear. As pointed out above, kinetic studies suggest that the impact of thyroid hormones on lipid transport is mainly exerted through effects on the elimination of lipids from the circulation. This notion is compatible with the decreased lipolytic activity in post heparin plasma (PHLA, representing the sum of LPL and HL activities) from patients with hypothyroidism (Nikkilä & Kekki 1972, Tulloch et al. 1973, Pykälistö et al. 1976). In hyperthyroidism, on the other hand, reports on PHLA are contradictory (Kirkeby 1968, Arons et al. 1972, Nikkilä & Kekki 1972, Tulloch et al. 1973). These data are also inconclusive regarding the influence of thyroid hormones on the separate activity of LPL and HL, since the activities of these enzymes were not selectively measured. There are, however, some data that suggest that thyroid hormone deficiency might be accompanied by lowered activities of both LPL (Pykälistö et al. 1976) and of HL (Krauss et al. 1974). Changes in the activity of LCAT in these patients (Lacko et al. 1978) further indicated that thyroid hormones might be of great importance for the intravascular metabolism of lipoproteins in man.

AIMS OF THE PRESENT STUDY

The purposes of the present investigations were

- to characterize the plasma lipoprotein patterns in patients with hypothyroidism and hyperthyroidism, and to relate the degree of dyslipoproteinemia to the levels of thyroid hormones
- to study the effects of thyroid hormone deficiency on the composition of the different lipoprotein fractions
- to investigate the influence of thyroid hormones on the activities of three key enzymes in the intravascular metabolism of lipoproteins: lipoprotein lipase (LPL), hepatic lipase (HL) and lecithin: cholesterol acyl-transferase (LCAT)
- to delineate the roles of LPL, HL, and LCAT for the development of the lipoprotein disturbances in thyroid dysfunction
- to define possible differences between primary and secondary (pituitary) forms of hypothyroidism with regard to the development of dyslipoproteinemia.

METHODOLOGICAL ASPECTS

Experimental designs

Both longitudinal and crosssectional studies were performed. Although more laborious, the longitudinal design offers certain advantages: interindividual variations are eliminated and separate control groups are generally not necessary. Furthermore, it is possible to register the time course for the effects of interventions, e.g. hormone replacement, and to investigate the relationship between the effects of intervention on different variables. To analyze, in a more general manner, the relations between thyroid hormone concentrations, lipoprotein levels, and enzyme activities over the entire spectrum of thyroid function, the crosssectional approach is preferable, since it can deal with a more polarized study population. For the interpretation of statistical correlations in terms of causal relationships and mechanisms, information from both approaches have been essential.

We have studied 4 types of thyroid dysfunction: patients with overt primary hypothyroidism (II, III), subclinical primary hypothyroidism (I), secondary hypothyroidism (VI) and hyperthyroidism (IV), who have been compared to a group of euthyroid subjects (I). The effects of treatment leading to euthyroidism were registered (II, III, IV, VI). Also, the metabolic response to experimental hyperthyroidism, induced by triiodothyronine administration to healthy subjects, has been recorded (V).

Thyroid function and thyroid hormone effects

The laboratory diagnosis of primary thyroid hypofunction is generally based on elevated serum concentrations of S-TSH. Serum levels of thyroxine (S-T₄) rather than of triiodothyronine (S-T₃) seem to determine TSH release (Silva & Larsen 1978, Ryder et al. 1980). During the early development of thyroid failure, an enhanced peripheral conversion of thyroxine to triiodothyronine seems to maintain adequate levels of S-T₃ (Chopra et al. 1978), and during this phase, elevated

levels of S-TSH can therefore be combined with normal levels of S-T3 (Bastenie et al. 1980). A gradually increasing level of S-TSH is a sensitive indicator of progressive failure of hormonal production from the thyroid gland, suitable for diagnostic purposes. However, increasing levels of S-TSH can not be expected to be paralleled by reciprocal changes in thyroid- dependent biological effects, which seem to be exerted by triiodothyronine through specific receptors (Oppenheimer et al. 1976). Close relationships have been found between estimated nuclear triiodothyronine receptor occupancy and heart rate, achilles tendon reflex relaxation time and plasma cholesterol levels (Bantle et al. 1980). Thyroxine is currently regarded as a "prohormone", and its metabolic potency can probably be accounted for by its conversion to triiodothyronine (Chopra et al. 1978). From these considerations it seems that S-T3 levels represent the best variable reflecting peripheral thyroid hormone effects in studies on metabolic processes. To minimize the risk that differences in peripheral conversion of thyroxine to triiodothyronine and/or reverse triiodothyronine would compromise S-T3 levels as indicator of biological thyroid effects, great care was taken to exclude patients with concomitant diseases or on drug treatment.

Thus, S-T3 levels were used to allocate the patients with primary thyroid hypofunction into two groups. One (overt hypothyroidism) consisted of patients who all had S-T3 concentrations below the lower reference limit. Naturally, these patients had elevated S-TSH and low S-T4 concentrations, and clinical signs of hypothyroidism. In the other group (subclinical hypothyroidism) all patients had S-T3 levels within the reference range, although the level of S-TSH was increased and that of S-T4 low or normal. In these patients, no or only minor signs of hypothyroidism were registered. Elevated concentrations of S-T3 (along with S-T4 and free thyroxine index) and clinical signs of hyperthyroidism were used as criteria of thyroid hyperfunction.

By the same reasoning S-T3 levels were also used to match patients with primary and secondary hypothyroidism with regard to peripheral thyroid hormone effects. Similarly, triiodothyronine rather than thyroxine was chosen to induce hyperthyroidism in healthy volunteers in order to get a rapid increase in S-T3 levels.

This system for allocation of the participating subjects provided well defined groups for the investigations. A greater variation in hormone profile might be anticipated in the patients with pituitary insufficiency. In this case we chose to keep cortisol concentrations as normal as possible and to withhold gonadal hormones during the observation period, in order to optimize the possibilities to evaluate the effects of thyroid hormones as such in these patients.

Analytical methods

Established methodologies were used for the determination of lipoprotein concentrations. The concentration of plasma triglyceride (P-TG) gives an acceptable estimate of the VLDL concentration, since VLDL is main carrier of triglyceride in plasma in fasting healthy subjects. It should be born in mind, however, that in the patients with overt hypothyroidism, the triglyceride component of IDL will contribute to a certain extent (<20%) to P-TG values. The HDL cholesterol concentration was determined after precipitation of apo-B containing lipoproteins and LDL cholesterol calculated according to the formula of Friedewald et al. (1972). Consequently, cholesterol in IDL particles will be included in the calculated LDL concentrations. Since the calculated LDL cholesterol values are valid only for samples with P-TG concentrations below 5 mmol/l, patients with excessive hypertriglyceridemia are specifically mentioned or excluded from the calculations. The lipoprotein distribution was always qualitatively checked by agarose gel electrophoresis. For more sophisticated analysis of lipoprotein density distribution and for the qualitative analysis of the composition of lipoprotein fractions, we used zonal ultracentrifugation. This is the only method to produce a continuous spectrum of lipoproteins with regard to density. In contrast to stepwise centrifugation techniques, the zonal

method easily reveals subfractions not normally present in plasma. This is of importance in an investigation of the lipoprotein pattern in hypothyroidism, where pathological lipoproteins frequently appear. Also the zonal method allows the quantitation of HDL subclasses, HDL₂ and HDL₃. By modifications (III) of the original technique (Danielsson et al. 1978), it was possible not only to quantitate all lipoprotein subclasses from one sample, but also to obtain representative samples from all particle populations, with minimal overlapping from adjacent fractions, for qualitative analysis.

Methods for determination of the activities of LPL and HL in postheparin plasma take advantage of differences in catalytic properties between these enzymes: LPL needs apolipoprotein C II for optimal activity, and is inhibited by protamin and by 1 M NaCl. The hydrolysis of emulsified triglyceride in the presence of 1 M NaCl is a generally accepted measure of HL activity, which was used also in this study. Selective measurements of LPL activity are more difficult to establish and require for example separation of enzymes by chromatography (Ehnholm et al. 1973) or inhibition of HL activity by specific antibodies (Huttunen et al. 1975). The method for selective LPL determination used in the present investigations (Nilsson-Ehle & Ekman 1977) takes advantage of another catalytic property of HL. This enzyme, in contrast to LPL, is active against triglyceride substrate only if this is emulsified by sonication in the presence of albumin. By modifying the emulsification procedure accordingly, it is possible to prepare an emulsion which LPL, but not HL, readily acts upon. It must be born in mind, however, that both enzymes remain intact during incubation with the so-called "substrate-specific" LPL method. Therefore, this assay is sensitive to impurities in the substrate triglyceride in another manner than immunological or separative techniques. As in all lipase assays, the presence of contaminating monoglyceride will affect the levels of enzymatic activity. In addition, however, the specificity of the "substrate-specific" systems rapidly deteriorates when the contamination by radioactively labelled monoglyceride exceeds 0.5% (Nilsson-

Ehle 1983), since HL rapidly attacks this contaminant regardless of mode of sonication. In the present studies, the radiopurity of the triglyceride substrate was frequently checked, and it was repurified when contaminants exceeded 0.2%.

The main difference between the two LCAT assays used is found in the choice of substrate. In the method described by Glomset & Wright (1964), heat-inactivated normal plasma is added as substrate. Changes in the esterification rate measured therefore reflect alterations mainly in the activity of the enzyme essentially independently of variations in the patient's lipoprotein pattern. In the method designed by Stokke & Norum (1971), on the other hand, the patient's own plasma constitutes the substrate, and the individual alterations in plasma lipoproteins will also influence the results. For example, increasing levels of HDL₂ are known to inhibit the LCAT reaction (Kostner 1978). In longitudinal studies, where variations in the HDL₂/HDL₃ ratio occur, changes in the endogenous esterifying ability (which is reflected in the Stokke and Norum assay) may therefore reflect alterations of the enzyme activity, of the substrate quality, or both. A combination of the two methods for determination of LCAT activity, however, gives a possibility to differentiate between these alternative interpretations.

RESULTS AND DISCUSSION

Thyroid hormones and plasma lipoprotein concentrations

Plasma lipid and lipoprotein concentrations in the different groups of patients (I) are summarized in Fig. 3. In short, primary hypothyroidism was accompanied by increased levels of plasma cholesterol mainly as a result of markedly elevated concentrations of lipoproteins in the low (LDL) and intermediate (IDL) density fractions, while there was only a tendency towards an elevation of the HDL fraction (I, II, III). P-TG levels were also slightly elevated, representing an increase of the VLDL fraction, and, to a smaller extent, the presence of IDL (I, II, III). Hyperthyroidism was associated with lowered concentrations of plasma cholesterol, which could be accounted for by decreased levels of LDL and HDL cholesterol (I, IV). P-TG concentrations were not significantly different from those of a control group.

It is evident from these data that the variations in plasma cholesterol with thyroid function are almost entirely due to changes in LDL cholesterol (I), while differences in HDL cholesterol levels, expressed in absolute terms, are of minor importance. However, in relative terms the HDL cholesterol levels in the hyperthyroid subjects was considerably (about 17%) lower than those of the euthyroid subjects (I).

The effects of changes in thyroid hormone levels, as observed during treatment of the different patient groups (II, III, IV) and after induction of experimental hyperthyroidism in healthy subjects (V), conformed excellently with the pattern from the crosssectional comparison of the groups. The relationships between lipoprotein concentrations and thyroid function levels are schematically represented in Fig. 4. These data confirm and extend earlier studies (see page 9), as well as more recent investigations (Abrams et al. 1981, Abrams & Grundy 1981, Lithell et al. 1981, Muls et al. 1982).

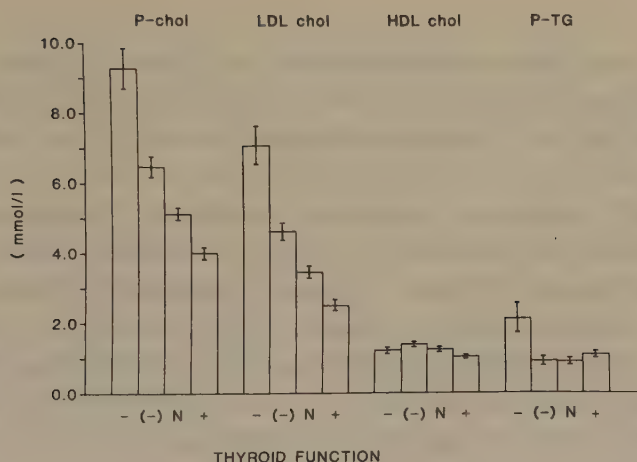


Fig. 3. Plasma concentrations of cholesterol, LDL-cholesterol HDL-cholesterol and triglyceride (P-TG) in patients with overt hypothyroidism -, n=20 ; subclinical hypothyroidism (-), n=12 ; euthyroid subjects N, n=17, and patients with hyperthyroidism +, n=21. Data are given as mean $\pm$ SEM.

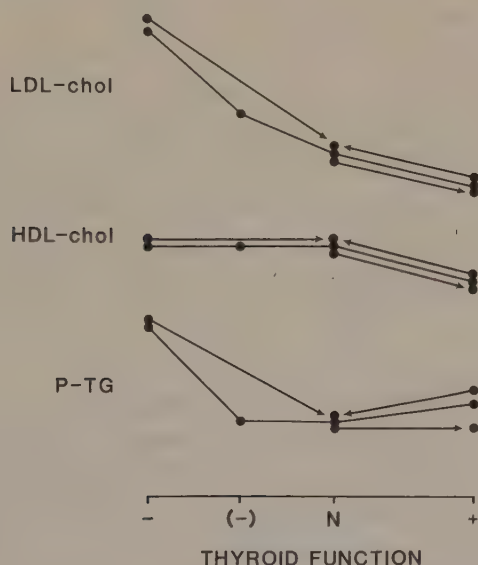


Fig. 4. Schematic representation of the influence of thyroid dysfunction on plasma concentrations of triglyceride, LDL cholesterol and HDL cholesterol. Symbols as in Fig. 3. Arrows indicate the effects of alteration of thyroid function through treatment of overt hypothyroidism and hyperthyroidism or through induction of experimental hyperthyroidism in euthyroid volunteers.

Of the different variables reflecting thyroid function (S-TSH, S-T3, S-T4, free thyroxine index), S-T3 turned out to give the closest correlations to lipoprotein concentrations (I, II, IV). As discussed above, this finding is well compatible with the view that S-T3 best reflects the biological effects of thyroid hormones. As pointed out by Bantle et al (1980), serum cholesterol levels are related to S-T3 concentrations in a non-linear fashion. In our patients (I), a similar non-linear relationship covering the full spectrum of thyroid function could be demonstrated between LDL cholesterol concentrations and S-T3 levels (Fig. 5). The significance of S-T3 concentrations for the disturbances in lipoprotein metabolism is further demonstrated by the positive correlation between the increase in S-T3 levels and the decrease in LDL cholesterol concentrations recorded during l-thyroxine substitution in hypothyroid patients (II).

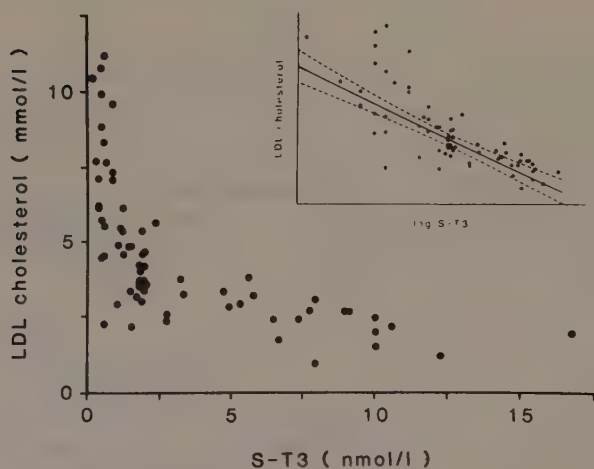


Fig. 5. Relationship between S-T3 and LDL cholesterol concentrations in 70 subjects with different levels of thyroid function. In semilogarithmic representation the correlation coefficient was -0.76 ($P < 0.001$). The broken lines indicate that 95 per cent confidence limits for the regression line. S-T3 reference range, $0.9-3.2$ nmol/l.

It is evident from Fig. 5 that lipoprotein alterations in subclinical forms of hypothyroidism may be of only minor degree. Changes in plasma cholesterol levels in these patients are therefore easily overlooked. Even a decrease in S-T3 concentrations within the reference range can, however, be accompanied by significantly increased concentrations of LDL cholesterol (I). The discrepancy between the present results and previous reports on patients with subclinical hypothyroidism (Nilsson et al. 1976, Kutty et al. 1978, Lithell et al. 1981), are probably due to the slightly more pronounced hypothyroidism exhibited by our patients: their mean S-TSH level was 61.3 mU/l, whereas 40 mU/l has been used as the upper limit in other investigations.

The lipoprotein distribution in hypothyroid patients was further investigated in detail using zonal ultracentrifugation (III). Overt hypothyroidism was associated with markedly increased concentrations of LDL particles, and considerable amounts of IDL particles could be demonstrated in plasma. L-thyroxine replacement resulted in regression of all these changes. Most prominently, the IDL fraction was dramatically reduced, and where still demonstrable, it had shifted in density and composition towards those of LDL. It is possible that this pattern may represent an impaired delipidation and impaired elimination of particles from the VLDL-LDL system.

Also the metabolism of plasma triglyceride was disturbed in thyroid dysfunction but to a smaller extent than that of plasma cholesterol. In most of our studies, we have taken P-TG as a measure of plasma VLDL concentrations. In hypothyroidism, where the metabolism of triglyceride is clearly disturbed, and the presence of IDL may preclude conclusions on VLDL concentrations from P-TG determinations, these measurements were extended by quantitation of VLDL by zonal ultracentrifugation (III).

The relation between S-TG and P-TG levels demonstrated a totally different pattern than that observed for S-TG and P-cholesterol (Fig 3, 4). Only patients with overt hypothyroidism had significantly increased concentrations of P-TG compared to euthyroid subjects (I). Previous longitudinal studies on S-TG in patients with thyroid dysfunction have generally shown a significant fall in S-TG levels after l-thyroxine replacement in hypothyroidism (Nikkilä & Kekki 1972, Tulloch et al. 1973, Rössner & Rpsenvist 1974, Ballantyne et al. 1979). These findings were confirmed in our patients with overt hypothyroidism (II, III), but we could not find any differences between euthyroid subjects and patients with subclinical form of hypothyroidism although the mean S-TSH level was clearly elevated (I). This discrepancy between overt and subclinical forms of hypothyroidism indicates that the metabolism of triglyceride-rich lipoproteins is impaired only in patients with severe thyroid hormone deficiency. It follows that a well defined patient material is of great importance for the evaluation of the impact of thyroid function on the metabolism of plasma triglyceride.

By zonal ultracentrifugation it was possible to demonstrate that the changes in P-TG levels were largely due to alterations in the VLDL fraction. Also, we could demonstrate a heterogeneity of the VLDL fraction, which frequently demonstrated "tailing", indicating the presence of denser particles in the VLDL peak. These "abnormal" fractions largely disappeared upon treatment (III). We interpret the denser VLDL particles as partly degraded VLDL in analogy with the IDL fraction as discussed above. These data from the zonal ultracentrifugation study are well compatible with the interpretation that deficiency of thyroid hormones leads to a defect catabolism of the triglyceride-rich lipoproteins. This view is also supported by the decreased ability to eliminate exogenous triglyceride demonstrated in patients with overt hypothyroidism (II). Although the improvement in triglyceride clearing after normalisation of thyroid function may partly depend on the decrease in P-TG levels, it is reasonable to assume that the increase in LPL activity (see below) occurring during l-thyroxine replacement, is

also of importance to facilitate the elimination of triglyceride after treatment.

Although no significant difference in P-TG levels was noted between the hyperthyroid patients and the euthyroid subjects (I), a moderate decrease in P-TG levels was recorded during treatment of hyperthyroidism (IV). These results illustrate the usefulness of a combined approach with longitudinal and cross-sectional studies. The data, as discussed more in detail below, probably reflect a multiple impact of thyroid hormones on the metabolism of plasma triglyceride.

The influence of thyroid hormones on HDL cholesterol concentration is less clear-cut. As pointed out earlier, only minor alterations in HDL cholesterol can be demonstrated in the hypothyroid patients, while in hyperthyroidism, a more consistent decrease in HDL cholesterol levels is at hand. Again, these changes were completely reversed by treatment (IV). These observations are consistent with recent investigations on patients with hypo- (Lithell et al. 1981, Abrams & Grundy 1981, Hylander & Rosenqvist 1982) and hyperthyroidism (Abrams & Grundy 1981, Muls et al. 1982).

During the last few years, considerable interest has focused on the concentrations and proportions of the HDL subclasses: the "negative risk" for coronary heart disease ascribed to HDL seems to be accounted for mainly by the HDL₂ subfraction (Miller et al. 1981). A few recent studies using selective quantitation of HDL₂ and HDL₃ indicate that HDL₂ is increased in hypothyroid patients (Brook et al. 1981, Aviram et al. 1982, Lisch et al. 1982), whereas the HDL subclass distribution in hyperthyroidism is controversial (Aviram et al. 1982, Lisch et al. 1982). Our hypothyroid patients had a selective increase in the HDL₂ subfraction, which normalized on treatment (III). In pilot studies of hyperthyroidism we have so far noted a clear, selective reduction of the HDL₂ level (Fig 6). The observation that HDL₂, but not HDL₃, is responsive to metabolic alterations is in agreement with findings in a variety of clinical and experimental conditions (Belfrage et al. 1977,

Ekman et al. 1981, Stubbe et al. 1982). This patterns is consistent with alterations in the interconversion of HDL₂ and HDL₃ (see Fig.2), which would have a greater impact on the relative concentration of the minor HDL₂ fraction.

The lack of correlation between S-T3 and total HDL cholesterol concentrations does not exclude an effect of thyroid hormones on HDL metabolism. For example, it is possible that systematic studies on HDL subfractions would reveal a relationship between S-T3 levels and the HDL₂ subfraction. As discussed more in detail below, thyroid hormones seem to affect HDL concentrations by several differently regulated mechanisms.

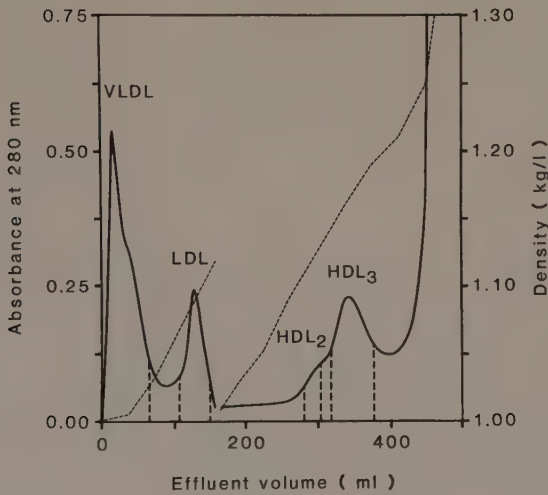


Fig.6. Lipoprotein profile obtained by zonal ultracentrifugation of plasma from a female patient with hyperthyroidism (S-T3 = 9.0 nmol/l, ref.range 0.9 - 3.2 nmol/l)
Full line indicates absorbance at 280 nm.
Dotted lines indicate density gradient.

It is often stated that patients with hypothyroidism of pituitary origin, in contrast to patients with primary hypothyroidism, have no or only minor disturbances of the lipoprotein concentrations. The thyroid hypofunction in patients with pituitary insufficiency is frequently less pronounced since these patients are in general closely supervised, and signs of thyrotropin deficiency are detected at an early phase (Hershman 1980). Since there is a close relationship between S-T3 levels and the degree of dyslipoproteinemia in primary hypothyroidism (I), it seems necessary to take into account the S-T3 levels for quantitative comparison of lipoprotein concentrations in primary and secondary hypothyroidism. Such an analysis clearly showed that there was no qualitative or quantitative differences in the lipoprotein patterns between these two forms of hypothyroidism (VI). Furthermore, thyroid hormone replacement induced a similar reduction of total plasma and LDL cholesterol levels in both groups of patients (II, VI). Thus, the alleged differences in lipoprotein concentrations between primary and secondary hypothyroidism can most likely be attributed to differences in thyroid hormone concentrations.

Lipoprotein lipase activity in thyroid dysfunction

LPL catalyzes the initial, rate-limiting step in the hydrolysis of triglyceride in lipoprotein particles. This process is essential for delivery of energy to tissues. It also results in the formation of low density lipoprotein particles from the triglyceride-rich lipoproteins, VLDL and chylomicrons.

Several factors influence the rate of the LPL reaction (Nilsson-Ehle 1982). The flux along the delipidation pathway is dependent on the concentrations of P-TG, since the enzyme is not saturated at physiological P-TG levels. The LPL reaction may also be influenced by alterations in the levels of activating and inhibiting apolipoproteins. Best known, however, is the active hormonal regulation of the enzyme. For example, its activity is higher in females than in males; insulin and glucocorticosteroids increase and catecholamines

decrease the activity of LPL in adipose tissue. The hormonal regulation can theoretically occur at several sites of control, e.g. the synthesis of enzyme protein; the activation of the enzyme occurring before or in conjunction with its secretion from the parenchymal cell; the transfer to its site of action at the endothelial cells; and finally the degradation of the enzyme.

Earlier studies have implicated a role for thyroid hormones in the regulation of LPL activity. Total postheparin lipolytic activity is decreased in hypothyroid patients (Nikkilä & Kekki 1972, Tulloch et al. 1973, Pykälistö et al. 1976), while results in hyperthyroidism are contradictory (Kirkeby 1968, Arons et al. 1972, Nikkilä & Kekki 1972, Tulloch et al. 1973). LPL activity in postheparin plasma, measured by specific methods, has been reported to be low (Lithell et al. 1981) or unchanged (Krauss et al. 1974, Abrams et al. 1981) in hypothyroid patients. In one recent study no changes were recorded during treatment for hyperthyroidism (Abrams et al. 1981). Reduced LPL activity has also been found in adipose tissue (Pykälistö et al. 1976, Lithell et al. 1981) and skeletal muscle (Lithell et al. 1981) in hypothyroid patients.

By comparison of groups of patients with different levels of thyroid function (I) we could demonstrate that LPL activity in postheparin plasma was significantly decreased only in patients with overt hypothyroidism, while patients with subclinical hypothyroidism did not differ from euthyroid subjects (Fig. 7). This finding is supported by the significant increase in LPL activity during l-thyroxine substitution in patients with overt hypothyroidism (II, III). Together with the data reported by Lithell et al. (1981), this demonstrates that a separation of patients with various degrees of thyroid hypofunction is necessary to detect the association between thyroid hormones and LPL activity. Patients with hyperthyroidism had normal LPL activities, and neither treatment of hyperthyroidism (IV), nor induction of experimental hyperthyroidism in healthy subjects (V) affected the activity of LPL.

The decrease in LPL activity in hypothyroidism may theoretically be due to changes in S-T3 and/or S-TSH. In rats, Robinson & Wing (1970) have demonstrated that LPL activity in adipose tissue is influenced by TSH. From the present investigations it can be concluded that, at least in man, the increased levels of TSH are not critical for the decreased activities of LPL in hypothyroid patients, since similar changes in enzyme activities were found in primary and secondary forms of thyroid hypofunction (II, III, VI).

It is also evident from our longitudinal studies that there are no immediate effects of thyroid hormone treatment on LPL activity. Administration of triiodothyronine, in doses leading to increasing S-T3 and reduced LDL cholesterol concentrations, to patients with overt hypothyroidism (II) as well as to healthy subjects (V), did not induce any significant changes in the activities of LPL after 48 h. Neither were there any consistent elevations of the activity of LPL during the initial phase of a standard replacement with l-thyroxine in patients with overt hypothyroidism (III). The slowly increasing LPL activities in these patients confirm and extend the observations of Pykälistö et al. (1976). Together these data demonstrate a clear discrepancy between the influence of thyroid hormones on LPL and HL, respectively.

The mechanisms behind the low activities of LPL in hypothyroidism can only be speculated on. Theoretically, effects on the synthesis of enzyme protein, on post-translational modification, on the secretion rate from the parenchymal cell, and on the binding to the glucosaminoglycan receptors of the endothelial cells may be involved (cf Pykälistö et al. 1976, Nilsson-Ehle 1982). However, the rather weak statistical relationships between S-T3 levels and LPL activities emerging in our studies, as well as the lack of rapid effects of triiodothyronine administration, makes it less likely that thyroid hormones would promote the activation-secretion step: for example, glucose and insulin, enhancing this particular step in

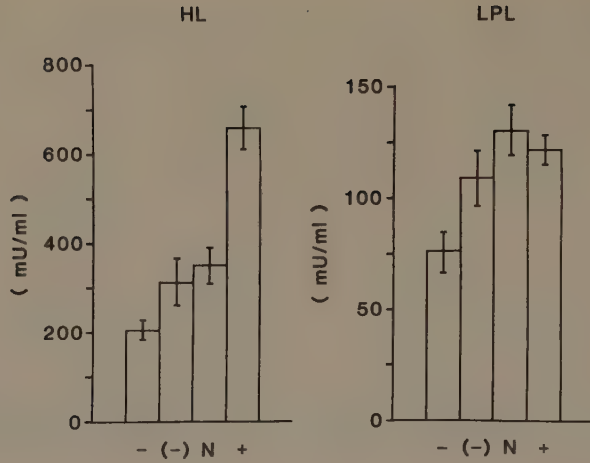


Fig.7. The activities of hepatic lipase (HL) and lipo-protein lipase (LPL) in patients with overt hypothyroidism -, n=20; subclinical hypothyroidism (-), n=12; euthyroid subjects N, n=17 and patients with hyperthyroidism +, n=21. Data are given as mean+SEM.

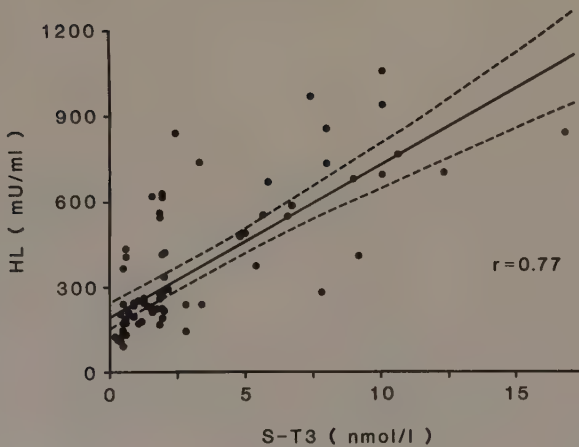


Fig.8. Relationship between S-T3 concentrations and hepatic lipase activities in 70 subjects with different levels of thyroid function. Correlation coefficient, 0.77 ($P<0.001$). The broken lines indicate the 95 per cent confidence limits for the regression line.

enzyme regulation, lead to a rise in enzyme activity within hours (Nilsson-Ehle et al. 1975, Garfinkel et al. 1976). Our tentative interpretation is therefore that the rise in LPL activity represents a non-specific effect of triiodothyronine on protein synthesis (Tata & Widnell 1966). An interesting possibility is, however, that the increase in enzyme activity would be explained by a prolonged extracellular half-life of the enzyme due to an altered binding to the glucosaminoglycans at the endothelium: the metabolism of glucosaminoglycans is known to change in hypothyroidism (Wegelius 1971).

It should be mentioned that attempts to further elucidate the relationship between thyroid function and LPL activity in experimental animals so far do not seem to give information of relevance for human pathophysiology. The hypothyroid rat, for example, shows increased levels of LPL activity in adipose tissue (Rosenqvist et al. 1981), and, as pointed out above, TSH as such seems to affect the activity of LPL in the rat, but not in man. The reasons for these species differences remain to be clarified.

Hepatic lipase activity in thyroid dysfunction

According to current hypotheses on lipoprotein metabolism (Fig. 2), HL takes part in the elimination of cholesterol from the circulation through its action as a phospholipase, acting on the HDL₂ particles during their passage through the liver (Kuusi et al. 1979 a, Jansen et al. 1980, Nikkilä et al. 1982, Rao et al. 1982). In analogy with LPL, HL is considered a secretory protein, which is synthesized in the hepatocytes and then transferred to its site of action at the endothelial cells in the liver sinusoids. Comparatively little is known about the regulation of HL activity. Females seem to have lower values than males (Huttunen et al. 1976). No significant changes with age have been reported (Huttunen et al. 1976). Oxandrolone (Ehnholm et al. 1975 b) and progesterin (Tikkanen et al. 1981) are both reported to increase and oestrogens (Tikkanen et al. 1982) to decrease the activity of HL. Weight reduction in

obese patients does not affect HL activity (Sörbris et al. 1981). Severe liver disease (Sauar et al. 1978) and renal failure (Applebaum-Bowden et al. 1979) are associated with lowered HL activity.

In 1974, Krauss et al. reported that the mean value for protam resistant lipase activity (i.e., HL) in postheparin plasma in four hypothyroid women was significantly decreased. Some years later, it was demonstrated that experimentally hypothyroid rats released significantly less lipase activity on liver perfusion than did controls (Hülsman et al. 1977, Jubelin et al. 1978). An effect on HL activity in patients with thyroid dysfunction has recently been reported by Abrams et al. (1981), who found decreased values for the activity of HL in hypothyroid patients and a slightly, but not significantly higher HL activity in hyperthyroid patients before than after treatment.

The data in the present investigations, using strictly defined groups of patients, consistently demonstrated a close relationship between thyroid hormone levels and the activity of HL over the full spectrum of thyroid function (I, II, IV). Of the different variables used to estimate thyroid function, S-T3 again turned out to give the best values in statistical evaluations of the relationship between thyroid function levels and HL activity (I). The stepwise higher levels of S-T3 recorded in the four groups of subjects with overt and subclinical hypothyroidism, euthyroidism and hyperthyroidism (I) were accompanied by similarly higher HL activities (Fig. 7). Analysis of individual values of all the participating subjects revealed a highly significant correlation between S-T3 concentrations and HL activities (Fig. 8). The coefficient of correlation was not improved after logarithmic transformation of either variable. This finding demonstrates that thyroid hormone levels are of importance for the activity of HL over the full spectrum of S-T3 concentrations. The activities of HL in patients with secondary hypothyroidism were not different from those of matched patients with primary hypothyroidism (VI), demonstrating that TSH is of little

importance also for this lipase activity.

Further support for the relation between thyroid hormone levels and HL activity was obtained in the longitudinal studies on hypo- and hyperthyroid patients (II, III, IV). The differences in HL activities before and after therapy were in both cases highly significant, and the changes in S-T3 levels during treatment were closely correlated to the alterations in HL activity. These strong relationships between S-T3 and HL indicate a direct effect of thyroid hormones on HL. This impression is further strengthened by the rapid effects of triiodothyronine intake on HL activity. Administration of triiodothyronine, in doses large enough to elevate the concentrations of S-T3 significantly, was followed by a rapid, significant increase in HL activity in patients with overt hypothyroidism (II) as well as in euthyroid subjects (V). A more rapid response for HL than for LPL was also seen during standard replacement with l-thyroxine to patients with overt hypothyroidism (III).

It therefore seems justified to conclude that the influence of thyroid hormones on the activity of HL is more direct than that on the activity of LPL (cfp 27). The mechanisms for such direct effects remain unclear. Thyroid hormones probably affect liver cells by multiple mechanisms rather than by a solitary mode of action (Sterling 1979). Effects on cell membrane and mitochondria may lead to primary initiating actions, while effects of binding of thyroid hormone to nuclear receptors may be evident only after a lag period. Besides, there is the possibility that thyroid hormones may influence HL activity at a later point in the processing of the enzyme, e.g. by altering the binding properties on glucosaminoglycans at the endothelial cell surface.

Lecithin: cholesterol acyltransferase in thyroid dysfunction

LCAT catalyzes the transfer of fatty acids from lecithin to cholesterol (Norum et al. 1982). Most of the esterified cholesterol found in plasma is formed by the action of LCAT. The enzyme circulates associated with HDL particles. One of its functions seems to involve the processing of free cholesterol and phospholipid which are transferred to HDL from the surface film of triglyceride-rich lipoproteins during their degradation. Part of the cholesteryl ester formed is incorporated into the core of the HDL particles; this process seems associated with the transformation of the smaller HDL₃ to larger HDL₂ particles (Patsch et al. 1978). A proportion of the esterified cholesterol is redistributed back to particles in the VLDL - LDL system (Fielding & Fielding 1981). Thus, a normal LCAT activity is of prime importance for the disposal of the surface components of the triglyceride rich lipoprotein particles. Since continuous removal of the surface film is a prerequisite for the complete lipolytic degradation of the core triglyceride, an impaired LCAT reaction will result not only in accumulation of phospholipid and free cholesterol, but also in hypertriglyceridemia (Norum et al. 1982).

LCAT is activated by apolipoprotein A I (Norum et al. 1982), while it is inhibited by high concentrations of HDL₂ (Kostner 1978) and of other cholesteryl ester acceptor lipoproteins (Fielding & Fielding 1981). Consequently, the endogenous esterifying ability depends not only on the enzyme activity but also on the concentration and composition of the HDL substrate as well as on other factors in plasma.

Information on hormonal influence on LCAT is scarce and interpretation of available data is difficult due to the various modes for expression of enzyme activity. Earlier data on the effects of thyroid hormones on LCAT (Adelkopfer et al. 1974, Lacko et al. 1978) indicate that the activity of the enzyme as such in hypo- and hyperthyroid patients is not different from normal since the (molar) cholesterol esterification rate is unchanged. Since serum cholesterol

levels differ widely between these groups of patients, this would lead to discrepancies in the fractional (%) cholesterol esterification rate; actually, this has been reported in one study on patients with thyroid dysfunction (Lacko et al. 1978). An effect of triiodothyronine on LCAT in hypothyroid rats has also been reported (Rosenqvist et al. 1981). As pointed out earlier (p¹⁷) the cholesterol esterification rate, as determined by the Glomset & Wright method, mainly reflects the activity of the enzyme as such. The Stokke & Norum assay also includes influence of changes of substrate and other plasma factors, and therefore reflects the actual, endogenous esterifying ability of the individual.

The present results (III, IV) demonstrate that the cholesterol esterification rate was not changed in hyperthyroidism, while it was moderately decreased in patients with overt hypothyroidism. The endogenous cholesterol esterifying ability, however, was increased in hyperthyroidism and decreased in overt hypothyroidism. The decrease recorded in these hypothyroid patients was greater than could be explained by alterations in the enzyme activity as such.

Together these data indicate that substrate alterations are of major importance for the LCAT reaction in all patients with thyroid dysfunction, and that the amount or activity of the enzyme proper is affected only in severe thyroid hormone deficiency. The proposed substrate effects are in good agreement with the altered concentrations of inhibitory HDL₂ in hypothyroidism (III) and hyperthyroidism (p 24) as demonstrated by zonal ultracentrifugation.

As pointed out above, overt hypothyroidism is accompanied by a moderately decreased activity of the enzyme as such. In this respect the influence of thyroid hormones on LCAT activity shows obvious similarities to that on LPL, which is also affected only in overt hypothyroidism. A further resemblance between LCAT and LPL activities is seen in the longitudinal experiments. During routine l-thyroxine replacement, both enzyme activities increased more slowly than the activity of HL, and normalized only after 12 weeks (III).

PROPOSED MECHANISMS FOR PLASMA LIPOPROTEIN ALTERATIONS IN THYROID DYSFUNCTION

Hypothyroidism

The increased concentrations of plasma cholesterol and tri-glyceride in hypothyroidism seem to result from defect removal rather than from increased production. Thus, kinetic cholesterol transport studies in hypothyroid patients have shown only minor alterations in cholesterol synthesis and intestinal absorption (Abrams & Grundy 1981). Similarly, synthesis of VLDL tri-glyceride seems to be essentially normal in hypothyroidism (Nikkilä & Kekki 1972). Kinetic studies have demonstrated an impaired elimination of VLDL-TG (Nikkilä & Kekki 1972) and a decreased catabolic rate of LDL particles (Walton et al. 1965) in hypothyroid patients. Such data are compatible with a defect in LDL receptor action, which has been reported in one hypothyroid patient (Thompson et al. 1981). The LDL receptor defect probably explains the increase in LDL concentrations.

It is obvious, however, that changes in the intravascular metabolism of lipoproteins are also of major pathophysiological importance for the disturbances of lipoprotein metabolism, and that alterations in LPL, HL, and LCAT activities contribute to the typical profiles of plasma lipoproteins in thyroid hypofunction (Fig. 9). We propose that decreased LPL activity (along with defect removal of lipoprotein surface components) leads to an impairment of the catabolism of the triglyceride-rich lipoprotein particles. This leads to hypertriglyceridemia, reflected in the zonal ultracentrifugation profile as an accumulation of VLDL proper, of partly degraded VLDL particles and of comparatively triglyceride-rich IDL particles.

Besides the LDL receptor elimination pathway, the elimination route for cholesterol via HDL to the liver also seems to be impaired. The LCAT activity is moderately lowered, and the HL activity, necessary for efficient cholesterol and phospholipid removal from HDL in the liver, is markedly decreased. This is

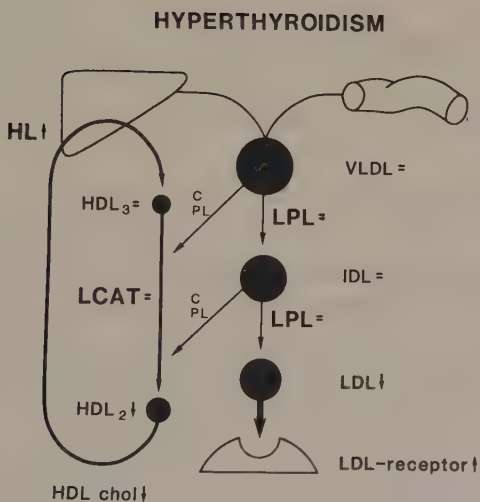
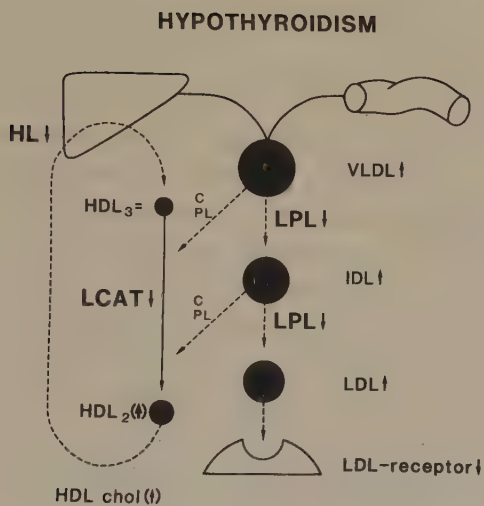


Fig. 9. Hypothetical models for intravascular lipoprotein metabolism in hypothyroidism and hyperthyroidism. C = unesterified cholesterol, PL = phospholipid.

counterbalanced by a decreased input of cholesterol into the HDL system, and the net content of cholesterol in the HDL system will therefore not be markedly affected. However, the pronounced changes in the HDL subclasses, with a relative increase in HDL₂, emphasize the pathophysiological importance of the lowered activity of HL. We suggest (II) that the decreased activity of HL can indeed contribute to the accumulation of lipoprotein particles in the IDL fraction. The IDL fraction can, as a matter of fact, be regarded as the result of a back-up in the flux of surface components from the VLDL - LDL system through HDL to the liver. The significance of such a mechanism is indicated by comparison with conditions associated with an isolated impairment of LPL or LDL receptor activity, which lead to accumulation of triglyceride-rich lipoproteins or LDL, respectively, but not of IDL (Fredrickson et al. 1967).

In short, we propose that decreased activities of HL, LPL and LCAT lead to an impairment, at multiple interacting sites, in the elimination routes for cholesterol and triglyceride from plasma in hypothyroid patients. Along with an alteration of the LDL receptor function, these enzyme mediated effects would largely explain the plasma lipoprotein changes seen in hypothyroidism.

Hyperthyroidism

Kinetic studies demonstrate only minor changes in the synthesis of cholesterol (Abrams & Grundy 1981), while the production of VLDL-TG is increased (Nikkilä & Kekki 1972) in hyperthyroid patients. The removal of triglyceride from the circulation (Nikkilä & Kekki 1972), the catabolism of LDL cholesterol (Walton et al. 1965 b) and the excretion of neutral steroids (Abrams & Grundy 1981) seem to be increased in hyperthyroidism. The main effect of excess of thyroid hormones on the transport of lipids therefore seems to be to promote their elimination from the circulation.

In vitro studies have shown that triiodothyronine can increase the number of LDL receptors in cultured fibroblasts (Chait et al. 1979). An enhanced elimination of LDL particles via

the LDL receptor pathway may therefore explain the lowered levels of LDL cholesterol in hyperthyroid patients.

The activity of LPL in postheparin plasma is not different from normal in these patients. Since LPL is not saturated at the levels of plasma triglyceride usually seen in hyperthyroid patients, the removal system can process an increased input of VLDL-TG, as may occur in hyperthyroidism (Nikkilä & Kekki 1972), and an increased flux along the VLDL degradation pathway can be accommodated with normal or only slightly elevated plasma triglyceride levels (Fig 9).

The surface components, released at an increased rate during the accelerated degradation of VLDL through the LPL reaction, can readily be accepted by the HDL fraction in hyperthyroid patients. The markedly increased activity of HL enhances the elimination of surface components in the liver and provide a HDL_2/HDL_3 ratio which is favourable for the LCAT activity. We suggest that the hepatic elimination of cholesterol is accelerated more than the input of cholesterol to HDL, providing an explanation for the low HDL cholesterol concentrations in these patients. This is in agreement with the finding of a selective reduction of the HDL_2 subclass seen in zonal ultracentrifugation (Fig 6, Lisch et al. 1982).

In short, we propose that the increased activity of HL is of major importance for the enhanced elimination of cholesterol from the circulation in hyperthyroid patients. Along with alterations in LDL receptor activity, the elevated HL activity would largely explain the changes in plasma lipoproteins seen in hyperthyroidism.

GENERAL SUMMARY

1. Plasma lipoprotein concentrations, the activities of three key enzymes in the intravascular metabolism of lipoproteins and the elimination rate of exogenous triglyceride were studied in patients with hypothyroidism and hyperthyroidism before and after treatment leading to euthyroidism.
2. Alterations in thyroid function affect the activities of hepatic lipase (HL), lipoprotein lipase (LPL) and lecithin: cholesterol acyltransferase (LCAT) but in different ways. Low levels of thyroid hormones are associated with decreased activities of all three enzymes, while increased concentrations of thyroid hormones are accompanied by elevated activity of HL but not of LPL or LCAT. Besides quantitative alterations in LCAT activity, changes in the lipoprotein substrate are of great importance for the endogenous cholesterol esterifying ability, which is decreased in hypothyroidism and increased in hyperthyroidism.
3. Plasma lipoprotein metabolism in hypothyroidism is characterized by an impaired degradation of triglyceride-rich lipoproteins. Together with a defect elimination of LDL particles via specific LDL receptors, this leads to an increased concentration of lipoprotein particles in the LDL and the IDL fractions and of partly degraded VLDL particles. Also the elimination of cholesterol via the HDL system to the liver is impaired. This is reflected in an elevation of the concentration of the HDL₂ subclass, although the total HDL cholesterol concentration is normal or only moderately increased.
4. Plasma lipoprotein metabolism in hyperthyroidism is characterized by an essentially normal degradation of triglyceride-rich lipoproteins. The cholesterol esterifying ability is increased. An increased activity of HL will facilitate the elimination of cholesterol from the HDL system. Along with an increased activity of the LDL

receptor pathway, this will lead to decreased concentrations of cholesterol, mainly in the LDL, but also in the HDL fraction.

5. Of the different variables used to reflect thyroid hormone function, (S-TSH, S-T₄, S-T₃, and free thyroxine index) S-T₃ turned out to give the best correlations to plasma lipoprotein concentrations and enzyme activities. This is in agreement with the view that triiodothyronine is responsible for the biological effects of thyroid hormones. Moderate but distinct disturbances of lipoprotein metabolism appear also in patients with moderate thyroid dysfunction, as in subclinical hypothyroidism.
6. There are no fundamental differences in the disturbances of lipoprotein metabolism in primary and secondary forms of hypothyroidism. The notion that the hypercholesterolemia in hypothyroidism of pituitary origin is less pronounced can probably be explained by the milder forms of thyroid hormone deficiency usually seen in secondary compared to primary forms of hypothyroidism.
7. Hyperthyroidism offers a possibility to study the effects on lipoprotein metabolism of a selective increase of HL activity. The findings in these patients are consistent with the concept that HL is a major determinant of HDL concentrations in man.

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RELATIONS BETWEEN THYROID FUNCTION, HEPATIC AND LIPOPROTEIN
LIPASE ACTIVITIES, AND PLASMA LIPOPROTEIN CONCENTRATIONS

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ABSTRACT

Lipoprotein concentrations and activities of lipoprotein lipase (LPL) and hepatic lipase (HL) were measured in 70 subjects with thyroid function ranging from overt hypothyroidism over subclinical hypothyroidism and euthyroidism to hyperthyroidism.

In parallel with serum T_3 ($S-T_3$) concentrations increasing from low in hypothyroidism to high in hyperthyroidism there were gradually higher HL activities over the full spectrum of thyroid function, accompanied by decreasing levels of total and low density lipoprotein (LDL) cholesterol. High density lipoprotein (HDL) cholesterol was lower ($P < 0.05$) in hyperthyroidism than in euthyroidism but not significantly changed in the hypothyroid groups. HL was correlated to $S-T_3$ ($r=0.77$, $P < 0.001$), LDL cholesterol to $\log S-T_3$ ($r=-0.76$, $P < 0.001$), and LDL cholesterol to $\log HL$ ($r=-0.55$, $P < 0.001$).

The activity of LPL was decreased ($P < 0.001$) in overt hypothyroidism compared to euthyroidism but, in contrast to HL, the activity of LPL was not increased in hyperthyroidism. The plasma triglyceride (P-TG) concentration was elevated ($P < 0.01$) in overt hypothyroidism but not significantly changed in subclinical hypothyroidism or in hyperthyroidism. The LPL activity was correlated to $\log S-T_3$ ($r=0.45$, $P < 0.001$), P-TG to $\log S-T_3$ ($r=-0.37$, $P < 0.01$) and P-TG to $\log LPL$ activity ($r=-0.71$, $P < 0.001$).

Our results demonstrate that thyroid hormones influence HL and LPL activities in different ways, suggesting different mechanisms of action. Changes in HL activity seems to be one important mechanism for the disturbance of cholesterol metabolism in thyroid dysfunction while the thyroid hormone influence on LPL seems to be of importance mainly for the disturbance in triglyceride metabolism.

When patients with hypothyroidism are treated with l-thyroxine an increase in lipoprotein lipase (LPL) and hepatic lipase (HL) activities has been found to parallel the normalization of their elevated total and LDL cholesterol levels (Pykälistö et al 1976, Lithell et al 1981, Abrams et al 1981, Valdemarsson et al 1982). In hypothyroid patients under treatment we also found the increase in S-T₃ to be significantly correlated to the increase in HL activity and to the decrease in LDL cholesterol as well (Valdemarsson et al 1982). These results indicate that LPL and HL is of pathogenic importance for the dyslipoproteinemia in hypothyroidism. However, the nature of a possible general association between thyroid hormone concentrations, lipase activities, and lipoprotein levels can not be determined from effects of treatment of hypothyroidism only, and data on lipase activities in hyperthyroidism are inconclusive (Nikkilä & Kekki 1972, Tulloch et al 1973, Abrams et al 1981). Therefore, we investigated these associations in a cross-sectional study of 70 subjects with various thyroid function states ranging from overt hypothyroidism over subclinical hypothyroidism and euthyroidism to hyperthyroidism.

MATERIAL AND METHODS

Patients: 53 (consecutive) patients and 17 ostensibly healthy subjects (doctors, technicians, students) were investigated after giving informed consent. The investigation was approved by the Ethical Committee of the University of Lund, Sweden. The subjects were allocated into four groups according to their thyroid function: Overt hypothyroidism (Group 1) with clinical signs of hypothyroidism, elevated serum levels of TSH (S-TSH) and S-T₃ below our lower reference limit; subclinical hypothyroidism (Group 2) with elevated S-TSH and S-T₃ within our reference range; healthy subjects (Group 3); and hyperthyroidism (Group 4) with elevated S-T₃. It is unlikely that the minor differences between the groups in mean body weight, age and sex distribution would significantly influence our conclusions from data on lipase activities (Huttunen et al. 1976) or lipoprotein concentrations (Barclay 1972). The substitution effects in 14 patients with overt hypothyroidism have been reported elsewhere (Valdemarsson et al 1982). Their

pretreatment values were included in Group 1.

All patients with increased S-TSH concentrations had elevated titers of circulating thyroid antibodies.

None of the subjects was on any drugs or diets known to influence serum lipids or thyroid function. Clinical and laboratory signs of diabetes or liver or kidney disease were absent.

Analytical methods: Methods for determination of thyroid hormone levels, activities of HL and LPL and lipoprotein concentrations have been described elsewhere (Valdemarsson et al. 1982).

Statistical methods: Coefficients of correlation and regression lines for different variables were calculated by the least square method by a computerized program. Data were also analyzed after transformation in the following ways: $\log.x$ vs. y , $\log.y$ vs. x , $1/x$ vs. y , $1/y$ vs. x , $\sqrt{x}$ vs. y , $\sqrt{y}$ vs. x and $1/x$ vs. $1/y$. Multiple regression analyses were performed by standard techniques (Draper & Smith 1966).

RESULTS

The mean values for the thyroid function variables in the patients with subclinical hypothyroidism (group 2) were intermediate between those of the patients with overt hypothyroidism (group 1) and those of the euthyroid subjects (group 3) and significantly different from both (Table 1). The thyroid function values in the hyperthyroid patients (group 4) were well separated from those of the healthy subjects (Table 1).

Table 1 also summarizes the mean values of lipoprotein concentrations, the activities of HL and LPL and the elimination rates of exogenous triglyceride. There was a continuous decrease in P-cholesterol concentrations from group 1 to group 4, the levels in all groups being significantly different from each other. A similar pattern was found for LDL cholesterol, while HDL cholesterol differed only in the hyperthyroid patients, who had 17% lower group mean concentration than the euthyroid subjects. The P-triglyceride level and IVFTT were significantly changed only in overt hypothyroidism.

The group mean values of HL activities (Table 1) gradually increased from markedly reduced levels in severe hypothyroidism to clearly elevated levels in hyperthyroid patients. In contrast, LPL activity was clearly lowered only in severe hypothyroidism, while the activities in subclinical hypothyroidism and in hyperthyroidism were not different from those of the euthyroid subjects.

The differences between the groups described for HL and LPL activities and for the lipoprotein concentrations were significant also when the values of the women were calculated separately to eliminate the possible influence of different sex distribution in the groups.

Table 2 summarizes the correlations between S-T₃ concentrations, HL and LPL activities, and lipoprotein concentrations calculated for the individuals in all groups together. Of the various transformations tested the logarithmic transformation was found to give the best adaptation to a rectilinear relationship throughout.

Very high correlations were found between S-T₃ and S-T₄ ($r = 0.95$, $\underline{P} < 0.001$) and between S-T₃ and FTI ($r = 0.97$, $\underline{P} < 0.001$). S-T₃ was found to give slightly better correlations to lipoprotein concentrations and enzyme activities than S-T₄ and FTI.

The total and LDL cholesterol concentrations were strongly correlated ($r = 0.98$, $\underline{P} < 0.001$). The coefficient of correlation between S-T₃ and LDL cholesterol increased from -0.60 to -0.76 after logarithmic transformation of S-T₃ (Fig 1). The correlations between S-T₃ or log S-T₃ and HDL cholesterol were weak or insignificant (Table 2). The coefficient of correlation for S-T₃ vs. P-triglyceride was -0.17, increasing to -0.37 ($\underline{P} < 0.01$) after logarithmic transformation of S-T₃.

A highly significant correlation was found between S-T₃ and HL activity ($r = 0.77$, $\underline{P} < 0.001$) (Fig 2) without improvement after logarithmic transformation. The correlation between S-T₃ and LPL activity was weaker and evident only after logarithmic transformation of S-T₃ (Table 2).

Total serum and LDL cholesterol levels were both strongly correlated to HL but more weakly to LPL without significant improvement after transformation (Table 2). When related to HDL

TABLE 1

Thyroid function variables, hepatic lipase and lipoprotein lipase activities, lipoprotein concentrations and the intravenous fat tolerance test in 70 individuals, allocated into four groups according to thyroid function. Data are given as mean $\pm$ SE. Reference ranges are those routinely used in our laboratory. LDL cholesterol concentrations were calculated according to Friedewald et al (1977) and include IDL cholesterol.

	Mean age years	Mean weight kg	S-T3 (nmol/l)	FTI	TSH (mU/l)	HL activity (mU/mL)	LPL activity (mU/mL)	LDL-cho1 (mmol/l)	HDL-cho1 (mmol/l)	TG (mmol/l)	IVFIT (%)
Group 1 (n=20)											
7 men	47.6	74.8	0.46 ⁺ 0.04	17.2 ⁺ 0.9	122.2 ⁺ 23.4	204.3 ⁺ 21.1	75.6 ⁺ 9.0	7.09 ⁺ 0.55	1.22 ⁺ 0.09	2.15 ⁺ 0.43	2.76 ⁺ 0.20
13 women			< 0.001	< 0.001	< 0.025	n.s.	< 0.05	< 0.001	n.s.	< 0.025	< 0.001
overt hypothy			< 0.001	< 0.001	< 0.001	< 0.005	< 0.001	< 0.001	n.s.	< 0.01	
P vs group 2											
P vs group 3											
Group 2 (n=12)											
2 men	49.9	64.7	1.53 ⁺ 0.15	46.6 ⁺ 5.6	61.3 ⁺ 8.9	313.3 ⁺ 53.2	109.2 ⁺ 12.5	4.63 ⁺ 0.26	1.39 ⁺ 0.07	0.92 ⁺ 0.13	6.12 ⁺ 0.83
10 women			< 0.05	< 0.001	< 0.001	n.s.	n.s.	< 0.001	n.s.	n.s.	
subclin hypothy											
P vs group 3											
Group 3 (n=17)											
9 men	34.7	75.5	1.93 ⁺ 0.09	93.1 ⁺ 3.6	3.1 ⁺ 0.2	349.9 ⁺ 40.9	130.4 ⁺ 11.2	3.46 ⁺ 0.18	1.26 ⁺ 0.08	0.90 ⁺ 0.11	-
8 women											
euthyroidism											
Group 4 (n=21)											
5 men	40.9	59.6	7.99 ⁺ 0.71	445.3 ⁺ 33.8	1.1 ⁺ 0.1	659.7 ⁺ 47.7	121.7 ⁺ 6.8	2.52 ⁺ 0.17	1.04 ⁺ 0.06	1.10 ⁺ 0.09	6.48 ⁺ 0.51
16 women			< 0.001	< 0.001	< 0.001	< 0.001	n.s.	< 0.001	< 0.05	n.s.	
hyperthy											
P vs group 3											
Reference ranges			0.9 - 3.2		0 - 8	220 - 520	110 - 220	1.7 - 4.4	0.8 - 1.6	0.4 - 2.1	5.0 - 6.9

TABLE 2

Coefficients of correlation between S-T₃, lipoprotein concentrations and the activities of hepatic lipase and lipoprotein lipase in 70 individuals with thyroid function ranging from overt hypothyroidism to hyperthyroidism. a, $p < 0.001$; b, $p < 0.01$; c, $p < 0.05$; n.s. not significant.

	Serum cholesterol	LDL cholesterol	HDL cholesterol	Serum triglyceride	HL	LPL
S-T ₃	-0.61 ^a	-0.60 ^a	-0.26 ^c	-0.17 ^{n.s.}	+0.77 ^a	+0.28 ^c
log S-T ₃	-0.79 ^a	-0.76 ^a	-0.18 ^{n.s.}	-0.37 ^b	+0.76 ^a	+0.45 ^a
HL	-0.55 ^a	-0.52 ^a	-0.35 ^b	-0.12 ^{n.s.}		
log HL	-0.59 ^a	-0.55 ^a	-0.39 ^a	-0.17 ^{n.s.}		
LPL	-0.46 ^a	-0.43 ^a	+0.21 ^{n.s.}	-0.49 ^a		
log LPL	-0.55 ^a	-0.48 ^a	+0.22 ^{n.s.}	-0.71 ^a		

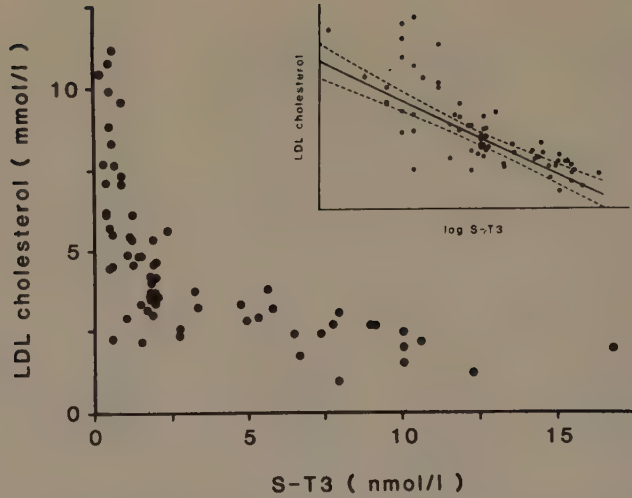


Fig 1. Relationship between S-T3 and LDL cholesterol concentrations in 70 subjects with different levels of thyroid function. In semilogarithmic representation the correlation coefficient was -0.76 ($P < 0.001$). The broken lines indicate the 95 per cent confidence limits for the regression line.

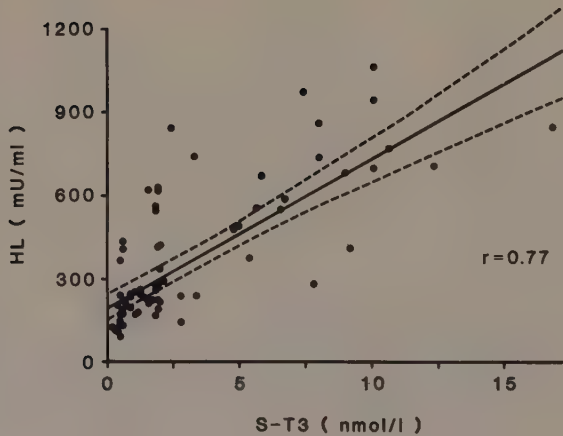


Fig 2. Relationship between S-T3 concentrations and hepatic lipase activities in 70 subjects with different levels of thyroid function. Correlation coefficient, 0.77 ($P < 0.001$). The broken lines indicate the 95 per cent confidence limits for the regression line.

cholesterol the coefficients of correlation were lower for both enzymes; however, the relationship between HDL cholesterol and HL activity was significant (Table 2). As expected, P-triglyceride concentrations were significantly correlated to the LPL activity, the coefficients of correlation increasing from -0.49 to -0.71 after logarithmic transformation of LPL values.

DISCUSSION

Thyroid function: Hypothyroidism is a graded phenomenon and can be subdivided into an overt form with clinical myxedema and subclinical forms with no or only mild clinical symptoms. The laboratory parameter that best reflects thyroid hormone action seems to be S-T₃ (Chopra et al. 1978; Bantle et al. 1980). A lowered S-T₃ as a line of demarcation between overt and subclinical hypothyroidism has been suggested by Bastenie (1980). Therefore we adopted the lower reference limit of our laboratory for S-T₃ as an arbitrary line of division between overt and subclinical hypothyroidism, and used the upper reference limit for S-TSH to differentiate the hypothyroid groups from normals, and an elevated S-T₃ as the criterion of hyperthyroidism.

The mean values in our grouped material were registered to permit comparisons with other materials. However, they suggested continuous changes with thyroid function of certain lipoprotein classes as well as of lipase activities over the entire range of thyroid function. The associations between these variables were therefore further elucidated by correlations of individual values for all participating subjects. In most cases the relationships between hormone concentrations and lipase activities or lipoprotein levels conformed better to the straight line after logarithmic transformation of hormone values. This is in accordance with the characteristically rectilinear relation seen between log concentrations and effects of most hormones. S-T₃ generally proved to be slightly stronger correlated to lipoprotein concentrations and lipase activities than S-T₄, FTI or S-TSH. This is consistent with the concept of triiodotyronine as the principal mediator of thyroid hormone action (Chopra et al. 1978).

Thyroid function and lipoprotein levels: The lipoprotein profiles of our patient groups are, in general, in agreement with earlier studies in more limited groups of patients with thyroid dysfunction (Walton et al. 1965; Rössner & Rosenqvist 1974; Agdeppa et al. 1979). It should be pointed out that the measure of LDL cholesterol (Friedewald et al. 1972) used in this study will also include IDL cholesterol. Most prominently, plasma LDL concentrations showed a continuous spectrum from elevated levels in severe hypothyroidism to concentrations in the lower reference range in hyperthyroidism. Correlations between individual values of $S-T_3$ and LDL cholesterol demonstrated a curvi-linear relation with a better adaptation to a straight line after logarithmic transformation of $S-T_3$. A similar relationship has been demonstrated between $S-T_3$ and total plasma cholesterol values (Ikejiri et al. 1978; Bantle et al. 1980).

Thyroid function and lipase activities: Lipoprotein lipase catalyzes the rate-limiting step in triglyceride removal from VLDL and chylomicrons, a process that is also associated with the formation of HDL cholesterol, whereas hepatic lipase seems involved in the removal of lipoprotein surface components (especially phospholipid and cholesterol), probably acting as a phospholipase using HDL as its substrate (Nilsson-Ehle et al. 1980). As pointed out above, both HL and LPL seem to be of significance for the dyslipoproteinemia in hypothyroidism whereas the role of these enzymes in hyperthyroidism is less well-known.

In a group of hypothyroid patients comprising overt as well as subclinical forms, LPL activity did not change significantly upon treatment with thyroxine (Abrams et al. 1981). However, our present results confirm a report indicating that LPL activity is low in overt but not in subclinical hypothyroidism (Lithell et al. 1981). Thus a separation of these forms of hypothyroidism seems necessary to detect an association between thyroid hypofunction and LPL activity. Our hyperthyroid patients did not differ from euthyroid subjects in LPL activity, which is consistent with the unchanged activity found after treatment for hyperthyroidism (Abrams et al. 1981). This pattern together with the weak correlation between $S-T_3$ and LPL values suggests an

indirect effect of T_3 on LPL activity e g via an effect on protein synthesis (Tata & Widnell 1966). Such a mechanism is also consistent with the delayed effect of thyroid hormone treatment on LPL activity in hypothyroidism (Valdemarsson et al. 1982).

In contrast, HL activity was related to S- T_3 concentrations over the full spectrum of thyroid function, with a three-fold difference between patients with hypo- and hyperthyroidism. The group mean value was significantly elevated in hyperthyroidism. A differently designed study by Abrams et al (Abrams et al. 1981; Abrams & Grundy 1981) showed only insignificant reduction of HL activity upon treatment for hyperthyroidism. However, a cross-sectional design with a euthyroid reference group may be more powerful in demonstrating an elevated HL activity in hyperthyroidism. The strong correlation between S- T_3 and HL values supports the view that T_3 has a direct effect on HL regulation (Hansson et al. 1982). This is consistent with the rapid response of HL activity to T_3 replacement in hypothyroidism (Valdemarsson et al. 1982).

Interrelations between hormone, enzyme and lipoprotein levels:
The metabolism of triglyceride-rich lipoproteins seems severely impaired only in overt hypothyroidism. These patients had elevated P-triglyceride levels, decreased LPL activities and impaired elimination of exogenous triglyceride. These variables were all statistically intercorrelated, and also correlated to S- T_3 levels. The correlation coefficient between log LPL and P-TG concentration was -0.71, corresponding to a coefficient of determination of 0.50; i.e., 50% of the variation in P-TG can be explained by differences in LPL activity. When S- T_3 was introduced as second variable in a multiple regression analysis, the coefficient of determination did not increase, demonstrating that S- T_3 was not correlated to P-triglyceride concentrations independently of LPL. These data support the view that LPL is one strong determinant of P-TG levels. The impact of T_3 on P-TG levels seems to be exerted mainly via the LPL mechanism.

Another group of correlations emerges around the metabolism of LDL. When hypothyroid patients were treated, the changes in

LDL cholesterol concentration, S-T₃ levels, and HL activities were intercorrelated (Valdemarsson et al. 1982). In the present investigation S-T₃ levels, HL activities and LDL cholesterol concentrations were all intercorrelated over the full spectrum of thyroid function states. In this case, the independence of the two possible determinants of LDL cholesterol, i.e., S-T₃ and HL activity, is difficult to establish by statistical methods. The coefficients of determination did not increase significantly when both S-T₃ and HL activity were included in the multiple regression analysis, a fact that is readily explained by the high correlation ($r=0.77$) between these two determinants. HL is considered to act upon HDL as its direct substrate (Nilsson-Ehle et al. 1980). It is possible that HL, under direct influence of T₃ enhances the elimination of cholesterol by facilitating the flow of surface components from VLDL-LDL via HDL to the liver. However, the strong correlation between log S-T₃ and plasma LDL levels ($r= -0.76$) indicates an additional mode of operation for T₃ in LDL regulation, such as a direct effect of T₃ on the LDL receptor (Chait et al. 1979).

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Reversal of decreased hepatic lipase and lipoprotein lipase activities after treatment of hypothyroidism

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Abstract. Plasma lipoprotein concentrations, activities of hepatic lipase and lipoprotein lipase in post-heparin plasma, and the removal rate of exogenous triglyceride were measured in fourteen patients with severe primary hypothyroidism before and after 4 months substitution therapy with L-thyroxine. Before treatment plasma LDL cholesterol concentrations were markedly increased while HDL cholesterol and plasma triglycerides were in the upper reference range. Thyroxine substitution led to a normalization of LDL cholesterol in all patients. Plasma triglycerides and HDL cholesterol decreased moderately. Hepatic lipase and lipoprotein lipase activities were initially reduced but increased significantly after treatment, by about 170% and 55%, respectively. The increase in hepatic lipase activities was significantly correlated to the increase in serum triiodothyronine levels and also to the reduction in LDL cholesterol concentrations. The decrease in LDL cholesterol was also significantly correlated to the increase in serum triiodothyronine concentration.

In two patients initially treated with triiodothyronine, the activity of hepatic lipase, but not that of lipoprotein lipase, increased after 24 and 48 h, while LDL cholesterol levels decreased substantially.

We suggest that the reduced activities of hepatic lipase as well as of lipoprotein lipase are important pathogenetic factors for the dyslipoproteinaemia occurring in hypothyroidism and that the low serum triiodothyronine concentration is of major importance for the alterations in lipid transport.

Key words. HDL cholesterol, LDL cholesterol, thyroxine, triiodothyronine, hepatic lipase, lipoprotein lipase, IVFTT, hypothyroidism.

Introduction

Among the most prominent metabolic consequences

of hypothyroidism are alterations in the plasma lipoprotein pattern [1–4]. The lipoprotein aberrations are mainly confined to the VLDL and especially the LDL classes, which are generally markedly increased, whereas the HDL fraction is essentially normal or only moderately elevated [5–7]. The alterations affect the core components (especially triglyceride) as well as the surface constituents (e.g. cholesterol and phospholipid) of the lipoprotein particles [6, 8]. A preferential increase in LDL triglyceride is often reflected in an accumulation of particles in the less dense subfractions (S_f 10–20), which are often referred to as intermediate density lipoproteins (IDL) [6]. The magnitude of the plasma lipoprotein alterations seems related to the severity of the hypothyroid state [9], and the lipoprotein pattern reverts towards normal after substitution with thyroxine [2, 4–6].

The mechanisms behind these alterations in lipoprotein concentrations and composition have not been settled, although several studies indicate that the lipoprotein alterations may be related to subnormal activities of key enzymes in lipoprotein degradation. These include lipoprotein lipase (LPL), which catalyses the rate-limiting step in the elimination of triglyceride from plasma [10], and hepatic lipase (HL), which is assumed to play a role in the removal of lipoprotein surface components by virtue of its phospholipase activity [11]. Both LPL and HL are released into the circulation by the intravenous injection of heparin. It has been repeatedly demonstrated that the total post-heparin lipolytic activity is reduced in hypothyroidism [12–15], but relatively few data are available on the activity of the individual enzymes. In groups of patients with hypothyroidism ranging from mild to severe forms the activity of HL, but not that of LPL, has been found to be reduced [16–18], and the HL activity increased after substitution with thyroxine [17, 18].

In the present study we have further penetrated the relationship between thyroid hormone levels, LPL and HL activities, and plasma lipoprotein concentrations in a well defined group of patients with severe hypothyroidism, who were studied before and after substitution to euthyroidism.

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Materials and Methods

Patients

Eight women (mean age 45.6 years, range 29–61) and six men (mean age 45.7 years, range 16–61) with primary hypothyroidism participated in the investigation and gave informed consent. All patients were clinically hypothyroid before substitution. The diagnosis was based on elevated S-TSH levels combined with subnormal serum thyroxine (S-T4) concentrations. Serum triiodothyronine (S-T3) concentrations were measured in eleven of the patients, and found to be subnormal as well (Table 1).

All patients had significantly elevated titres of circulating thyroid antibodies and were interpreted to have hypothyroidism as a consequence of autoimmune thyroiditis.

Table 1. Variables reflecting thyroid function in patients with primary hypothyroidism before and 4 months after start of substitution therapy with l-thyroxine. Data are given as mean $\pm$ SEM and range

	T3 (nmol/l)	T4 (nmol/l)	TSH (mU/l)
Before substitution	0.45 $\pm$ 0.05 (0.1–0.8)	22.4 $\pm$ 1.4 (10–25)	146 $\pm$ 33 (100–560)
After substitution	1.93 $\pm$ 0.15 (1.2–2.7)	108.9 $\pm$ 7.4 (74–157)	3.5 $\pm$ 0.6* (1–8)
Reference range	0.9–3.2	57–154	0–8
<i>n</i>	11	14	14

* One incompletely substituted patient has been excluded, see text.

No drugs known to influence serum lipids were prescribed. None of the patients suffered from diabetes mellitus. Clinical and laboratory signs of liver or kidney disease were absent.

After substitution all patients were relieved of their hypothyroid symptoms. S-T4 and S-T3 increased in all patients to normal values (Table 1). S-TSH decreased to 8 mU/l or less in all patients except in one, whose TSH concentration decreased only to 56 mU/l, as full thyroxine substitution could not be instituted due to symptoms of ischaemic heart disease.

The mean body weight of the female patients before and after substitution was 70 kg (range 61–82) and 69 kg (range 65–76), respectively, and of the male patients 86 kg (range 70–102) and 82 kg (range 69–92), respectively.

Experimental design

Blood sampling and functional tests were performed under strictly standardized conditions between 08.00 and 10.00 hours after the patients had been fasting for at least 10 h. Substitution with gradually increasing

doses of l-thyroxine (Levaxin, Nyegaard) was then started. The initial dose was 0.05 mg every second day and the final dose 0.15–0.20 mg daily. The variables reflecting thyroid function normalized after about 4 weeks and the investigations were repeated after about 4 months.

In two cases this regimen was preceded by the administration of 20 μ g triiodothyronine with 8 h intervals during 48 h.

Analytical methods

S-T3 was determined radioimmunologically (Amersham). S-T4 was measured by a competitive protein binding technique [19, 20]. S-TSH was determined by radioimmunoassay with a double antibody technique [21].

HDL-cholesterol was determined in the clear supernatant obtained after precipitation of VLDL and LDL by MgCl_2 and dextran sulphate [22]. Triglyceride and cholesterol were measured by enzymatic methods [23, 24]. LDL-cholesterol was calculated according to the formula $\text{LDL-cholesterol} = \text{P-cholesterol} - (\text{HDL-cholesterol} + 0.45 \times \text{P-triglyceride})$ [25]. The lipoprotein patterns were always checked by agarose gel electrophoresis.

Plasma samples for the determination of lipase activities were drawn 15 min after the intravenous administration of 50 U heparin (Vitrum, Stockholm, Sweden) per kg body weight [26]. The activities of hepatic lipase and lipoprotein lipase were determined by specific methods using tri- ^3H oleoyl glycerol as substrate [26].

The elimination rate of exogenous triglyceride was determined by the intravenous fat tolerance test (IVFTT) as described by Carlsson & Rössner [27]. 1 ml per kg body weight of 10% Intralipid (Vitrum, Stockholm, Sweden) was given and serum samples were drawn every 5 min for 40 min. The disappearance rate was calculated from a semi-logarithmic plot of light scattering index *v.* time and expressed as % per min.

Statistical calculations

The significance of differences was evaluated using Student's *t*-test. Correlations between different variables were analysed by linear regression analysis.

Results

Before treatment the mean plasma cholesterol level was 10.0 ± 0.7 mmol/l (mean $\pm$ SEM) with a marked increase in LDL cholesterol (7.8 ± 0.7 mmol/l). HDL cholesterol levels were in the upper reference range, the mean value being 1.34 ± 0.13 mmol/l (Fig. 1). One patient had a markedly increased plasma triglyceride concentration (13 mmol/l); the mean value for the rest of the patients was 1.66 ± 0.22 mmol/l.

After substitution with thyroxine the mean plasma cholesterol level (Fig. 1) decreased to 5.4 ± 0.2 mmol/l.

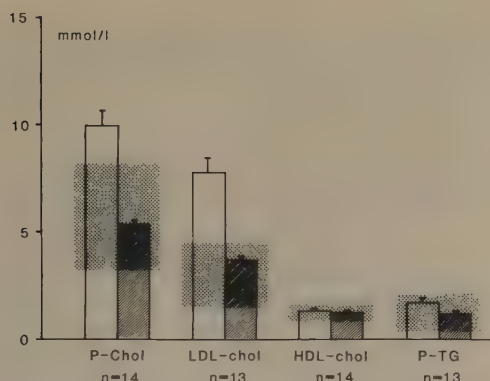


Figure 1. Plasma triglyceride and cholesterol, LDL cholesterol, and HDL cholesterol levels in fourteen patients with primary hypothyroidism before (open bars) and after 4 months on thyroxine substitution therapy (hatched bars). Data are given as mean $\pm$ SEM. Shaded areas indicate reference ranges. One patient with marked hypertriglyceridaemia has been excluded from the calculations of LDL cholesterol and triglyceride (see text).

($P < 0.001$). All patients reached values within the reference range. The reduction in plasma cholesterol could be accounted for almost entirely by a reduction in LDL cholesterol levels (Fig. 1) which decreased by about 50% to 3.7 ± 0.2 mmol/l ($P < 0.001$). The comparatively small reduction in HDL cholesterol to 1.19 ± 0.09 mmol/l (Fig. 1) was, however, statistically significant ($P < 0.025$). The single patient with marked hypertriglyceridaemia decreased in plasma triglyceride concentration from 13.0 to 2.9 mmol/l, whereas the rest of the patients had a reduction of the mean triglyceride concentration by about 25% to 1.17 ± 0.12 mmol/l ($P < 0.05$).

In all samples chylomicrons and pathological lipoprotein classes were absent, as judged by the electrophoretic pattern.

Following the substitution with thyroxine the activity of HL increased in all patients, and that of LPL in all but one (Fig. 2). The group mean values increased from 177.7 ± 24.8 to 483.5 ± 60.7 mU/ml for HL ($P < 0.001$), and from 73.8 ± 8.3 to 115.4 ± 10.1 mU/ml for LPL ($P < 0.005$). The elimination rate of exogenous triglyceride increased in all subjects, the mean value of the group being $2.79 \pm 0.32\%/min$ before and $5.24 \pm 0.47\%/min$ after substitution ($P < 0.001$).

The decrease in total plasma cholesterol, and in LDL cholesterol as well, was significantly ($P < 0.05$) correlated to the increase in S-T3 ($r = 0.58$ and 0.59 , respectively) but not to the increase in S-T4. There were no significant correlations between the changes in thyroid hormone concentrations and the changes in HDL cholesterol or triglyceride concentrations.

The increase in the activity of HL was significantly correlated ($r = 0.70$, $P < 0.01$) to the increase in S-T3 (Fig. 3) but not to that in S-T4. The increase in LPL activity was weakly but significantly correlated to the change in S-T4 ($r = 0.55$, $P < 0.05$) but not to that in S-T3. The increase in the elimination rate of exogenous triglycerides did not correlate significantly to the increase in S-T3 or S-T4. The increase in HL activity was significantly correlated ($r = 0.58$, $P < 0.05$) to the reduction in LDL cholesterol but not to the changes in HDL cholesterol.

In two of the patients thyroid hormone substitution was initiated with $20 \mu\text{g}$ T3 administered with 8 h intervals for a total of six doses. The therapy was then continued with T4, with subsequent stepwise increase as described above. The T3 administration increased the S-T3 levels substantially. The increase in S-T3 was associated with an increase in the activity of HL and a

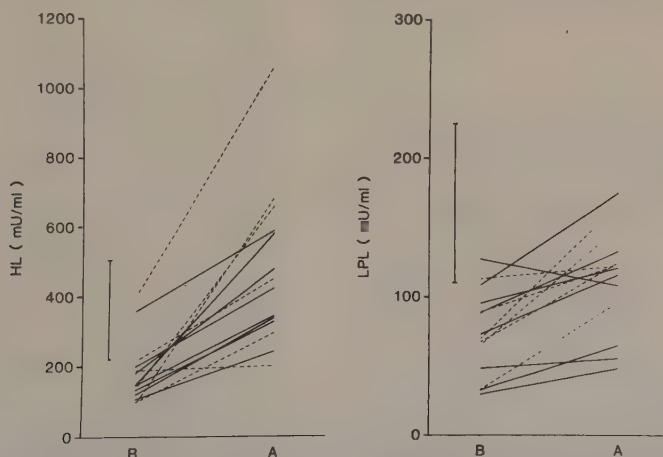


Figure 2. Activities of HL and LPL before (B) and after (A) 4 months of thyroxine substitution therapy in fourteen patients with primary hypothyroidism. Solid lines indicate females, dashed lines males. Vertical bars indicate reference ranges.

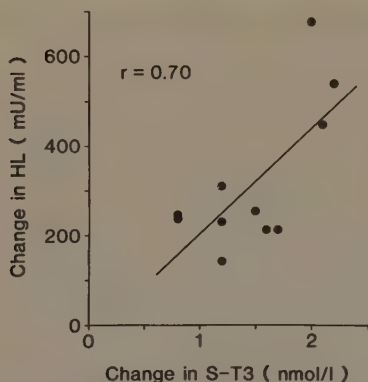


Figure 3. Linear regression of the increase in S-T3 and the increase in HL activity following substitution to euthyroidism in fourteen hypothyroid patients.

decrease in LDL cholesterol visible already 24 h after the first T3 dose and still more pronounced after 48 h, while there was no increase in LPL activity (Fig. 4).

Discussion

The term hypothyroidism based on an elevated S-TSH may be combined with a thyroid hormone function ranging from practically normal ('subclinical hypothyroidism') to very pronounced forms of the disease [28]. Evidently the degree of hypofunction is of importance for the impact of hypothyroidism on lipoprotein metabolism [9], and the selection of patients is there-

fore critical. The thyroid function seems to be better reflected by S-T3 or S-T4 than by S-TSH [29, 30]. Our patients form a well-defined group with severe hypothyroidism characterized by very low S-T4 and, when measured, subnormal S-T3.

The present study confirms the pattern of lipoprotein alterations in patients with hypothyroidism. In accordance with earlier studies [1-4], we found moderately elevated levels of plasma triglyceride and markedly increased concentrations of plasma cholesterol. Quantitation of HDL and evaluation of gel electrophoretic patterns demonstrated that the changes were mainly confined to the VLDL-LDL-system, with essentially normal levels of HDL. After adequate substitution the lipoprotein profile normalized and in all patients there was a substantial decrease in total and LDL cholesterol that was significantly correlated to the increase in S-T3 but not to that in S-T4. This emphasizes the functional importance of S-T3 [cf. 29] and contrasts to the smaller and less consistent changes in the lipoprotein profile found in other comparable investigations upon substitution of hypothyroidism [e.g. 17, 18]. The difference may be explained by the selection of patients, patients with mild hypothyroidism showing only a small decrease or even an increase in serum cholesterol levels upon substitution that normalized S-TSH [18].

In an attempt to elucidate the mechanisms behind these lipid changes in hypothyroidism we have monitored the clearance rate of triglyceride (IVFTT) and selectively measured the two major post-heparin lipases, i.e. LPL and HL activities, before and after substitution. The increase in triglyceride removal rate from the lower to the upper reference range, which

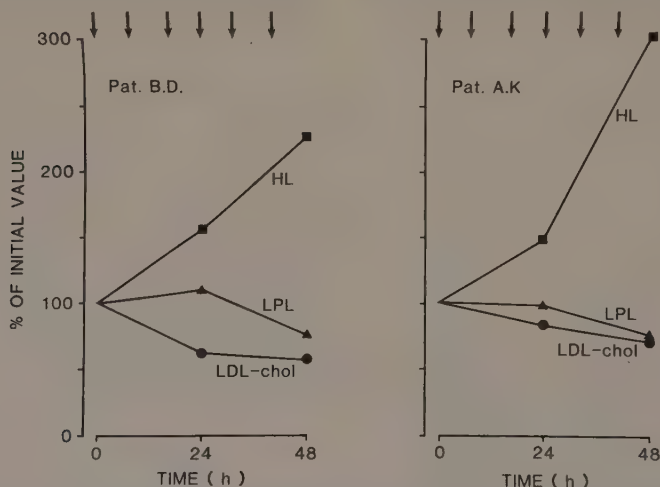


Figure 4. Changes in HL and LPL activities and LDL cholesterol concentrations following the administration of T3 in two hypothyroid patients. Each arrow indicates the oral administration of 20 μ g T3. In patient B.D. S-T3 increased from 0.5 to 1.9 nmol/l after 24 h, and to 2.7 nmol/l after 48 h. In patient A.K. S-T3 increased from 0.4 to 2.9 nmol/l after 24 h, and to 5.1 nmol/l after 48 h. HDL cholesterol concentrations were 0.7, 0.8 and 0.7 mmol/l in patient B.D., and 1.3, 1.0 and 1.1 mmol/l in patient A.K. at 0, 24 and 48 h, respectively.

might reflect an increased LPL activity and/or decreased plasma triglyceride level is consistent with earlier findings [5, 12, 13]. Similarly, the significant increase in LPL activities in post-heparin plasma is well compatible with recent data demonstrating that subnormal activities of LPL in adipose tissue [15, 31] and in skeletal muscle [31] in patients with severe hypothyroidism increase significantly after treatment. Data on LPL activity in post-heparin plasma of hypothyroid patients, however, are contradictory, and low [31] as well as normal [16, 17, 31] activities have been reported in different studies. Comparison of the selection of patients indicate that only in severe hypothyroidism, as studied in the present investigation, is LPL in post-heparin plasma decreased, whereas mild thyroid hypofunction does not seem to affect the LPL activity [17, 31].

It is well established that LPL activity in adipose and other tissues is hormonally regulated [10, 32]; the hormonal effects include rapid changes of enzyme activities that are not related to changes in protein synthesis (e.g. insulin, catecholamines) as well as long-term alterations which are dependant on protein synthesis (e.g. glucocorticoids) [32]. The lack of immediate effects on LPL activity in the patients who were initially treated with T3 indicates that the increase in LPL seen after full substitution of our patients may be a result of the positive effect of thyroid hormones on protein synthesis [33].

Another striking finding in the present study was the low activity of HL in thyroid hypofunction and the marked two- to three-fold increase in this enzymatic activity observed after substitution. These data confirm the observation of Krauss *et al.* [16] demonstrating low activities of HL in four patients with hypothyroidism. Abrams *et al.* [17] also found an increase in HL activity upon substitution of hypothyroidism that was demonstrable also in patients with mild hypothyroidism.

Our results are also consistent with studies in experimentally hypothyroid rats which release significantly less lipase activity on liver perfusion than controls [34, 35]. The regulation of HL activity has been the subject of comparatively few studies [10]. The considerable increase in HL activity after substitution and the significant correlation between the increase in S-T3 levels and HL activities indicates that thyroid function is an important determinant of HL activity. The rapid rise in enzyme activity after T3 administration suggests a direct effect of T3 on HL regulation.

The lipoprotein alterations in hypothyroidism thus reflect defects in the catabolism of the lipoprotein core components (moderate hypertriglyceridaemia, slow clearance of plasma triglyceride, and low LPL activity) as well as in the disposal of the surface film (cholesterol and phospholipid) of the native triglyceride-rich lipoproteins, leading to an accumulation of unusually light LDL (IDL) particles [6, 8]. The significant relationships between the changes in plasma and LDL cholesterol concentrations, S-T3 levels and HL activities

after treatment suggests that the impact of thyroid function on lipoprotein metabolism is largely mediated via alterations in HL activity. Recent studies on HL indicate that this enzyme has its physiological role in assisting in the disposal of the surface components by virtue of its prominent phospholipase activity [11]. It has been proposed that HL is active against these phospholipids only after their transfer from the VLDL-LDL system to the HDL system [11]. In the present study we found essentially normal HDL levels before treatment, and only a moderate reduction in HDL concentrations (measured as HDL cholesterol) was recorded parallel to the dramatic increase in HL activity during thyroxine treatment. We therefore suggest that an intact HL function, continuously modifying the HDL particles, is a prerequisite for the transfer of the surface components cholesterol and phospholipid from the VLDL-LDL system to the HDL system, and that the accumulation of LDL in the S_r 10–20 range in hypothyroid patients represents a back-up in the metabolic pathway of surface components from the triglyceride-transporting lipoproteins via HDL to the liver. However, our data are also compatible with the view [36] that HL may act directly upon IDL; in that case the dyslipoproteinaemia in hypothyroidism would be even more closely related to the subnormal activities of HL. It is also possible that part of the reduction in LDL cholesterol induced by thyroid hormones may be mediated via a stimulation of LDL cholesterol removal by LDL receptors [37].

Acknowledgments

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III

CHANGES IN PLASMA LIPOPROTEIN CONCENTRATIONS AND
COMPOSITION DURING REPLACEMENT THERAPY IN HYPOTHYROID
PATIENTS: RELATIONSHIP TO ALTERATIONS IN LIPOLYTIC
ENZYMES AND LCAT

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ABSTRACT

Plasma lipoproteins, including the HDL subfractions HDL₂ and HDL₃, were studied by zonal ultracentrifugation in 10 patients with severe hypothyroidism before and after substitution to euthyroidism. Besides lipoprotein concentrations and composition, the activities of lipoprotein lipase, hepatic lipase and lecithin: cholesterol acyl-transferase (LCAT) were measured.

Before therapy, LDL concentrations were increased and IDL was present in all subjects. The VLDL fractions was more heterogeneous than normally seen. A slight increase in HDL was totally accounted for by an increase in the HDL₂ subfraction. Lipoprotein lipase, hepatic lipase and LCAT activities were all low.

During replacement therapy all lipoprotein alterations reverted and had normalized within 14 weeks. The proportion of protein in IDL increased, and the relative amount of cholesteryl ester in HDL₂ decreased. The activity of hepatic lipase rose more rapidly than lipoprotein lipase and LCAT activities. A quantitative difference in the effects on LCAT activities measured with endogenous and exogenous substrate indicates that the impairment of cholesterol esterification in hypothyroidism is due to effects on the substrate as well as on the enzyme activity.

The data demonstrate that hypothyroidism is associated with alterations not only in the VLDL-LDL system, but also in the HDL system, and emphasize the role of disturbances in hepatic lipase activity for these lipoprotein changes.

Key words: low density lipoprotein, high density lipoprotein, zonal ultracentrifugation, lipoprotein lipase, hepatic lipase, lecithin: cholesterol acyltransferase, hypothyroidism, thyroxine

Abbreviations used in this paper:

HDL	= high density lipoprotein
HL	= hepatic lipase
IDL	= intermediate density lipoprotein
LCAT	= lecithin: cholesterol acyltransferase
LDL	= low density lipoprotein
LPL	= lipoprotein lipase
T ₃	= 3,5,3'-triiodothyronine
T ₄	= thyroxine
TSH	= thyrotropin
VLDL	= very low density lipoprotein
"VLDL ₂ "	= subfraction of VLDL in the higher density range of this lipoprotein class.

INTRODUCTION

Patients with overt hypothyroidism have markedly elevated concentrations of serum cholesterol. This elevation is mainly due to an increase in the low density lipoprotein (LDL) concentrations. There is also an accumulation of cholesterol-rich lipoproteins of intermediate density (IDL) (1 - 3), which are found only in negligible concentrations in normal subjects. Although less prominent, elevated concentrations of high density lipoprotein (HDL) cholesterol have been reported, mainly reflecting an increase in the HDL₂ subfraction (4 - 7). During replacement therapy the concentrations of LDL seem to normalize within three months, and a moderate, transient decrease in HDL-cholesterol has been described (8).

Data on the composition of lipoprotein classes in hypothyroidism are less abundant. Although not statistically significant, there is a tendency towards an enrichment of cholesteryl ester in the LDL and HDL₂ subfractions (7).

Several investigations have led to the conclusion that impaired activities of the key enzymes in the intravascular lipoprotein metabolism are of importance for the dyslipoproteinemia seen in hypothyroidism. Lipoprotein lipase (LPL) (4, 9 - 13), hepatic lipase (HL) (4, 9, 11, 13) and lecithin: cholesterol acyltransferase (LCAT) (14) activities are all low in hypothyroid patients, and increase towards normal

during replacement therapy (4, 10, 11, 14). Short-term experiments with triiodothyronine administration to hypothyroid patients (4) and normal subjects (15) have demonstrated that HL, but not LPL, responds rapidly to hormone administration. Little information is, however, available on the time-course for the increase of these enzyme activities during standard 1-thyroxine substitution of hypothyroid patients (cf 10).

The present investigation was undertaken to further study the relationship between lipase and LCAT activities and lipoprotein concentrations and composition in patients with hypothyroidism before and during replacement therapy. Zonal ultracentrifugation was employed since it is superior to step-wise ultracentrifugation methods with predetermined density cuts for the analysis of samples with qualitatively altered lipoprotein classes. Also, the zonal technique allows quantitation of the HDL subfractions, HDL₂ and HDL₃.

MATERIALS AND METHODS

Patients

Ten patients with hypothyroidism participated after giving informed consent. The investigation was approved by the Ethics' Committee of the University of Lund.

Five women and four men suffered from primary hypothyroidism, associated with increased levels of thyroid antibodies. Patient no 10 had secondary hypothyroidism without cortisol deficiency or signs of pituitary tumor. Clinical data are given in Table 1. Patient no 5 was treated with metoprolol after myocardial infarction. Patient no 6 took prazosin and metoprolol for essential hypertension. These medications were withdrawn during four days prior to the investigations. None of the patients had signs of other diseases, and no diets were prescribed.

All patients were severely hypothyroid (Table 1) and had serum thyroxine ($S-T_4$) concentrations below 25 nmol/l. Substitution therapy was given with increasing doses of l-thyroxine, starting with 0.05 mg every second day. The patients were re-investigated when they had been euthyroid for at least three months. They had all been relieved of their signs of hypothyroidism, and serum thyrotropin ($S-TSH$) and thyroid hormone levels had normalized in all patients.

TABLE 1

Clinical and hormonal data of hypothyroid patients before (B) and after (A) l-thyroxine replacement therapy. For thyroid hormones and TSH, the reference ranges used in our laboratory are indicated.

Patient number	Sex	Age (years)	Body mass index ^a	Body weight (kg)		TSH (mU/l)		S-T ₃ (nmol/l)		S-T ₄ (nmol/l)	
				B	A	B	A	B	A	B	A
1	M	54	28.7	83	80	>100	2	0.5	2.5	<25	77
2	F	62	33.7	82	76	>100	3	<0.5	2.5	<25	84
3	M	45	24.0	71	69	>100	2	<0.5	1.6	<25	114
4	F	30	21.9	61	65	>100	8	0.5	1.7	<25	95
5	M	57	26.9	91	85	>100	3	0.8	2.0	<25	110
6	M	35	31.2	101	98	>100	2	0.5	2.0	<25	114
7	F	66	28.3	80	74	69	2	<0.5	1.8	<25	143
8	F	40	24.7	68	67	>100	5	<0.5	1.6	<25	105
9	F	42	24.7	73	71	>100	1	<0.5	1.2	<25	107
10	F	65	30.0	74	73	6	1	0.6	2.5	<25	111
Mean		49.6	27.4	78.4	75.8		2.9		1.94		106.6
S.D.		12.9	3.7	11.6	9.8		2.1		0.45		18.21
ref range						<8		0.9-3.2			57-154

^a Body weight (kg)

Height² (m²)

Analytical Methods

TSH and thyroid hormones were measured by radioligand assays using reagents from MILAB, Malmö, Sweden. Triglycerides and cholesterol were analyzed by enzymatic kits from Boehringer Mannheim GmbH. HDL-cholesterol was measured after precipitation of VLDL, IDL and LDL by dextran sulfate and MgCl_2 (16). LDL-cholesterol was calculated according to the formula of Friedewald (17) modified to SI units, assuming an average molecular weight of 884 for plasma triglyceride; this estimate of LDL will also include lipoproteins of intermediate density. The plasma lipoprotein profile was also evaluated by agarose gel electrophoresis to verify the absence of chylomicrons.

LPL and HL were assayed by specific methods using tri- $[\text{}^3\text{H}]$ oleoylglycerol as substrate (18). Phosphatidyl choline (0.067 mg/ml) was used instead of lyso-phosphatidyl choline to emulsify the substrate.

LCAT activity was estimated by two methods: The technique described by Stokke and Norum (19) measures the endogenous cholesterol esterifying ability of plasma. The method of Glomset and Wright (20) uses pooled, heat-inactivated plasma as lipoprotein substrate. An intravenous fat tolerance test was performed as described by Carlsson and Rössner (21).

Zonal Ultracentrifugation

Plasma lipoproteins, including HDL subfractions, were separated in a Beckman Ti-14 zonal rotor run in a Beckman L-2-65B ultracentrifuge, by a modification of the method described by Danielsson et al (22). Fifteen g of plasma adjusted to a density of 1.295 was layered under a NaBr gradient (density 1.00 - 1.30). After centrifugation for 2h at 44.000 rpm, the rotor was decelerated to 3.000 rpm, and about 350 ml of the rotor content (containing VLDL, IDL and LDL) were collected in 7.5 ml portions. The gradient was then reconstituted and HDL₂ and HDL₃ separated by further centrifugation at 44.000 rpm for 22 h. The protein content of the collected fractions was monitored by spectrophotometry at 280 nm. The areas of the peaks of the respective lipoprotein classes were measured by planimetry. The absence of LDL in the HDL fraction and vice versa was verified by immunological measurements of apolipoprotein B and A-I (16).

The composition of lipoprotein classes was determined in representative pools from each lipoprotein fraction. Triglycerides and total cholesterol were assayed as above. Free cholesterol was measured by omission of cholesterol esterase from the reagent. Phospholipid was assayed as the phosphorus content of a lipid extract (23) and protein by the method of Lowry et al (24).

Statistics

Statistical significances of changes were evaluated by Wilcoxon's rank order test.

RESULTS

Replacement with l-thyroxine led to a significant decrease in plasma (P-) cholesterol concentrations (Table 2) which could be accounted for mainly by a fall in LDL cholesterol. The HDL cholesterol concentration decreased, however not significantly; the LDL-HDL ratio was reduced by about 50%. P-triglyceride concentrations decreased by about 35%.

The lipoprotein patterns of two representative patients (no 2 and 3), as studied by zonal ultracentrifugation, are shown in Fig 1. The most prominent features in the initial samples (Fig 1a, c) were relatively high LDL concentrations; the presence of an IDL peak, sometimes as a shoulder in the less-dense LDL region; increased asymmetry of VLDL with a relative increase of a denser VLDL subfraction ("VLDL₂"); and a comparatively high HDL₂/HDL₃ ratio.

Treatment resulted in regression of all these changes (Fig 1b, d), and the lipoprotein profiles were now similar to those observed in normal subjects (22). The amounts of LDL, IDL and "VLDL₂" were all markedly reduced (Fig 2), and IDL had virtually disappeared in patients No 5 and 9. The IDL fraction consistently had a slightly higher density after treatment. On substitution therapy, a selective decrease of HDL₂ was noted, resulting in a decreased HDL₂/HDL₃ ratio. We could not detect any consistent changes in the mean densities of the HDL subfractions.

TABLE 2

Plasma lipids and lipoproteins in hypothyroid patients before and after l-thyroxine replacement. HDL-cholesterol was measured after precipitation of apolipoprotein B-containing lipoproteins. LDL (including IDL) cholesterol was calculated according to Friedewald. Data are given as mean $\pm$ SEM.

	P-tri- glyceride (mmol/l)	P-chol- esterol (mmol/l)	P-LDL- choles- terol (mmol/l)	P-HDL- choles- terol (mmol/l)	LDL/HDL ratio
Before	2.12 $\pm$ 0.25	9.59 $\pm$ 0.74	7.61 $\pm$ 0.78	1.03 $\pm$ 0.12	8.4 $\pm$ 1.3
After	1.36 $\pm$ 0.16	5.19 $\pm$ 0.22	3.63 $\pm$ 0.84	0.95 $\pm$ 0.07	4.1 $\pm$ 0.4
Significance of difference	$\underline{P} < 0.01$	$\underline{P} < 0.01$	$\underline{P} < 0.01$	n.s.	$\underline{P} < 0.01$
Reference ranges	0.3 - 2.4	3.2 - 8.3	1.7 - 4.9	0.8 - 1.6	-

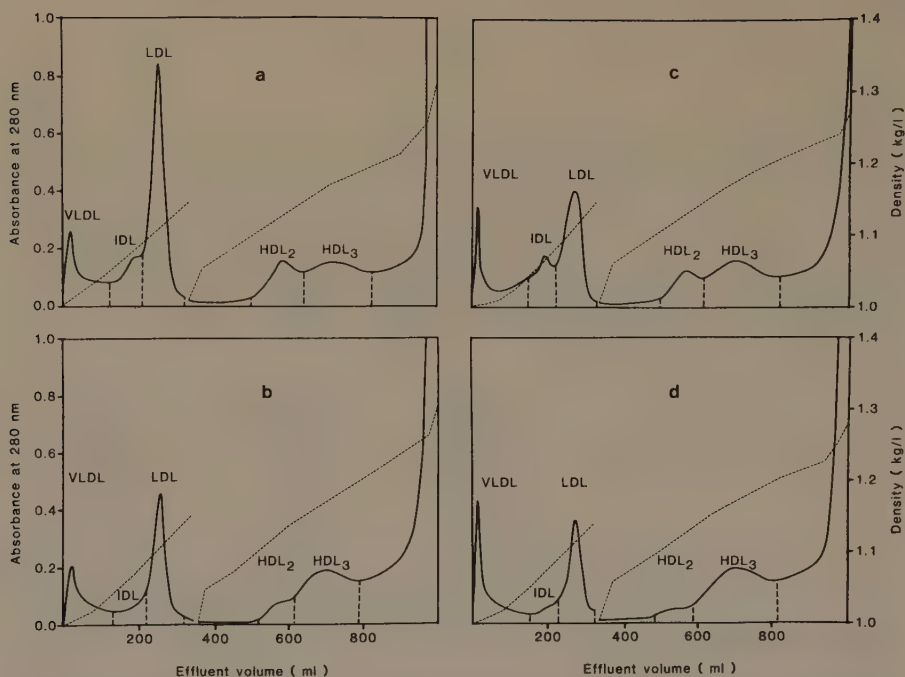


Fig 1. Typical samples of plasma lipoprotein patterns obtained by zonal ultracentrifugation before and after treatment for hypothyroidism. Panels a and c represents patients 2 and 3 before substitution and b and c the same subjects after therapy.

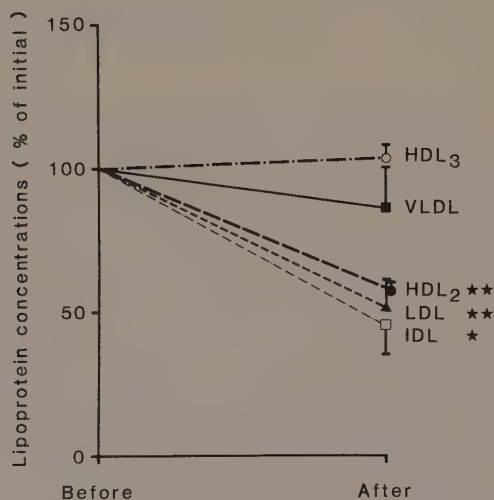


Fig 2. Mean changes in plasma lipoprotein concentrations, measured by zonal ultracentrifugation, after L-thyroxine substitution in ten hypothyroid patients.

* = $\underline{p} < 0.05$, ** = $\underline{p} < 0.01$.

The composition of the lipoprotein classes before and after treatment are shown in Table 3. The "VLDL₂" fraction contained less triglyceride and more phospholipid and cholesterol ester than normal VLDL. The IDL fraction differed from LDL mainly by its higher triglyceride content. Substitution therapy resulted in a relative increase of protein in the IDL fraction, mainly balanced by a decrease in triglyceride. Qualitative changes in LDL were smaller; however, there was a tendency towards a decrease in triglyceride contents. There were no qualitative changes in HDL₃; however, replacement therapy led to a significant decrease in the cholesterol ester content of HDL₂, and a corresponding increase of the protein component.

The activities of LPL and HL increased in all patients, and the mean value for both enzyme activities was significantly ($P < 0.01$) higher after treatment (Fig 3). The elimination rate of exogenous triglyceride rose from 2.60 ± 0.25 to 4.92 ± 0.45 %/min ($P < 0.01$). The fractional cholesterol esterification rate determined with the patient's plasma as substrate (Stokke-Norum method) increased by more than 120% during treatment. When blood donor plasma was used as substrate (Glomset-Wright technique) the rate increased by about 65%. The increase in esterification rate recorded with the Stokke-Norum method was significantly greater than that registered with the Glomset-Wright assay ($P < 0.01$).

In three patients (no 1 - 3) we followed the time-course for the changes in lipoproteins and enzyme activities during replacement therapy to full substitution (Fig 4). The low

TABLE 3 Composition of lipoprotein fractions (% w/w) before and after replacement therapy in hypothyroid patients. Data are given as mean $\pm$ SEM.

		Triglyceride	Cholesteryl ester	Free cholesterol	Phospho- lipid	Proteins
VLDL	before	53.7 $\pm$ 1.8	8.9 $\pm$ 0.9	5.4 $\pm$ 0.4	13.9 $\pm$ 1.1	18.1 $\pm$ 2.2
	after	59.4 $\pm$ 2.4	7.4 $\pm$ 0.8	4.8 $\pm$ 0.4	13.7 $\pm$ 1.4	14.6 $\pm$ 1.9
VLDL ₂	before ^{a)}	37.9 $\pm$ 3.2	15.6 $\pm$ 4.6	7.8 $\pm$ 0.1	20.3 $\pm$ 1.0	18.5 $\pm$ 3.1
IDL	before ^{b)}	18.0 $\pm$ 3.2	34.2 $\pm$ 2.5	9.1 $\pm$ 0.5	22.1 $\pm$ 0.5	16.7 $\pm$ 0.8
	after ^{b)}	15.2 $\pm$ 4.3	33.5 $\pm$ 3.2	8.3 $\pm$ 0.6	21.3 $\pm$ 0.6	21.8 $\pm$ 1.4
LDL	before	7.1 $\pm$ 0.5	39.8 $\pm$ 0.8	8.4 $\pm$ 0.3	22.4 $\pm$ 0.4	22.3 $\pm$ 0.5
	after	5.4 $\pm$ 0.8	41.6 $\pm$ 1.2	8.3 $\pm$ 0.5	21.7 $\pm$ 0.5	23.0 $\pm$ 1.0
HDL ₂	before	8.0 $\pm$ 0.8	19.9 $\pm$ 1.2	4.2 $\pm$ 0.3	25.7 $\pm$ 1.1	42.3 $\pm$ 1.4
	after	9.5 $\pm$ 1.4	15.3 $\pm$ 1.4 ^{d)}	4.2 $\pm$ 0.4	25.2 $\pm$ 0.8	45.8 $\pm$ 1.3
HDL ₃	before	4.9 $\pm$ 0.3	16.1 $\pm$ 0.6	2.1 $\pm$ 0.1	22.5 $\pm$ 0.9	54.5 $\pm$ 0.8
	after	5.2 $\pm$ 0.6	14.7 $\pm$ 1.0	2.1 $\pm$ 0.1	23.5 $\pm$ 0.8	54.6 $\pm$ 1.1

a) The composition of this fraction was measurable only in three patients after treatment

b) Represents data from eight patients who had a demonstrable IDL fraction also after treatment

c) Significantly ($P < 0.05$) different from initial value

d) Significantly ($P < 0.01$) different from initial value

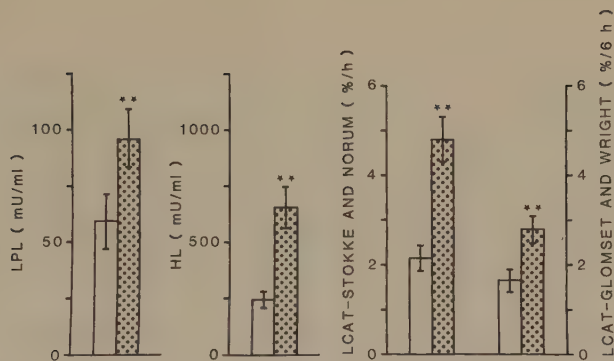


Fig 3. Activities (mean + SEM) of lipoprotein lipase (LPL), hepatic lipase (HL) and lecithin:cholesterol acyltransferase (LCAT) before and after substitution of hypothyroid patients. The LCAT activity was measured as endogenous cholesterol esterification rate (Stokke and Norum) and with exogenous plasma as substrate (Glomset and Wright). ** = $P < 0.01$.

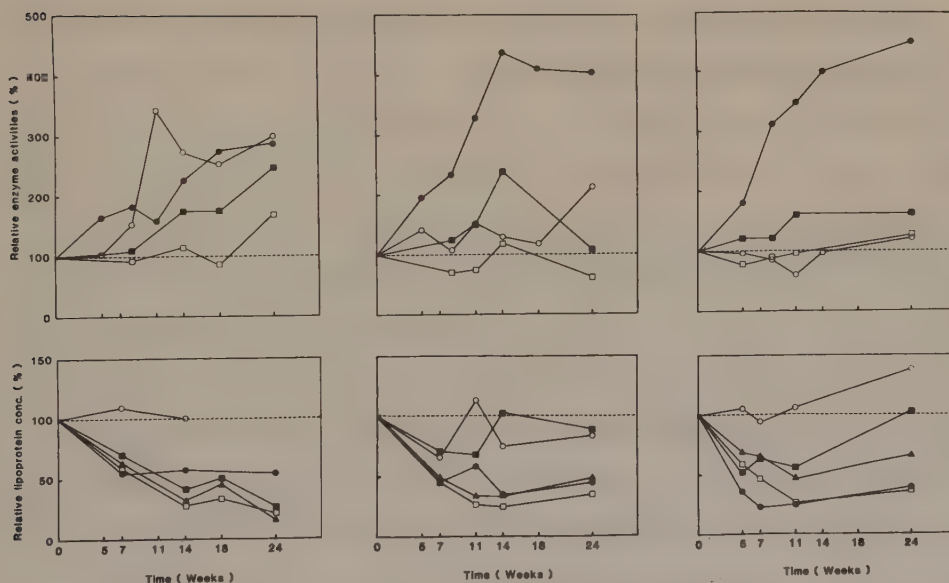


Fig 4. Time-course for changes in plasma lipids, lipoprotein fractions and lipolytic enzyme activities during substitution therapy. From left, the panels represent patients 1, 2 and 3. The upper parts of the panels represent the relative activities of LPL (open circles), HL (closed circles), LCAT by the Glomset-Wright technique (open squares) and by the Stokke-Norum technique (closed squares). The lower part demonstrate the relative concentrations of VLDL (closed squares), IDL (open squares), LDL (closed triangles), HDL₂ (closed circles) and HDL₃ (open circles), as quantitated by zonal ultracentrifugation.

initial activity of HL rose gradually to normal values. The increase in enzymatic activity was evident already at the first sampling occasion. The activities of LPL and LCAT, in contrast, increased more slowly and with a greater variability.

Consecutive zonal ultracentrifugations demonstrated a gradual decrease in "VLDL₂", IDL, LDL and HDL₂ during the first 14 weeks of replacement therapy (Fig 4). We could not find any consistent differences for the time-course of the alterations in these lipoprotein classes. The VLDL and HDL₃ classes showed no consistent changes. The composition of the various lipoprotein classes gradually changed towards to the pattern described in Table 3. Changes in the different components seemed to parallel each other.

DISCUSSION

The dyslipoproteinemia of overt hypothyroidism provides an interesting model for the study of lipoprotein metabolism. Earlier studies using step-wise centrifugation techniques (1-3,6,8,12), are, in general terms, compatible with the present data. However, as pathological lipoproteins are present, zonal ultracentrifugation is the method of choice for the separation of lipoproteins from hypothyroid patients. Specifically, the shift in densities of VLDL and IDL would not have been detected by step-wise ultracentrifugation.

The most marked qualitative alterations in hypothyroid patients were displayed in the VLDL-LDL system. All patients had a clearly demonstrable IDL fraction, which contained more triglyceride and was less dense than the LDL fraction; also, the VLDL fraction was more heterogeneous than normally seen. It contained particles with an increased density ("VLDL₂") which were partly depleted of triglyceride and enriched in cholesterol and phospholipid. It is likely that both these lipoprotein fractions represent partly degraded VLDL particles, occurring as a result of an impairment of the delipidation pathway of VLDL to LDL. In addition, the concentrations of normal LDL were elevated; this finding seems related to the low activity of the LDL receptor pathway in hypothyroidism (25).

The changes in HDL were confined to the HDL₂ subfraction. In agreement with earlier studies (5-7), the concentration of

this lipoprotein class was comparatively high, and it was enriched in cholesteryl ester. These results conform with the growing evidence (26) that the HDL₂, but not the HDL₃ sub-fraction, is subject to changes with alterations of the metabolic state. We suggest that the high concentration of HDL₂ in hypothyroid patients largely result from the low activity of HL, which is supposed to act as a phospholipase using HDL₂ as its substrate (27).

When replacement therapy was instituted, the qualitative and quantitative alterations in the lipoprotein classes all reverted. The changes in the lipoprotein pattern were completed in about 14 weeks which is consistent with the results of Hylander & Rosenqvist (8).

In accordance with earlier studies we found low activities of LPL (4, 9-13), HL (4, 9, 11, 13) and LCAT (14) in the hypothyroid patients. All increased on substitution with thyroxine. The increase in cholesterol esterification rate was most pronounced when measured with the patient's own serum as substrate. In the method of Glomset and Wright, using exogenous serum as substrate, the increase was smaller. This discrepancy indicates that the decreased cholesterol esterification rate in hypothyroidism is due to changes of both enzyme and substrate: a low activity of the enzyme as such seems combined with changes in the HDL substrate which impair the LCAT reaction. Since LCAT is inhibited by its product, HDL₂ (27), it is reasonable that the substrate effect is related to the high HDL₂/HDL₃ ratio in the hypothyroid subjects.

During substitution, we found a more rapid and marked elevation in HL activity than in LPL activity or cholesterol esterification rate. This finding is consistent with our earlier data demonstrating that only HL responds rapidly to T_3 administration (4, 15). Still, we found no differential changes in the plasma lipoprotein fractions during treatment. Specifically, we found no selective decline in HDL₂, especially its phospholipid component, during the initial part of therapy; such an effect might have been expected from present views on the function of HL. It is possible that other mechanisms operating in this complex system are masking the response to the altered enzyme activity at the specific time points used in this study. For example, rapid effects on the LDL receptor pathway may provide an additional means for an increased removal of cholesterol and phospholipids from plasma lipoproteins.

The changes in lipoprotein classes were evident during the early phase of therapy, before LPL and LCAT activities had started to rise. This indicates that the alterations in HL activity are of specific importance for the lipoprotein alterations in hypothyroidism. We suggest that the initial effects of substitution are exerted via effects on HL activity and the LDL receptor pathway. Increased LDL removal (25) seems responsible for the rapid fall in LDL concentrations, while the disappearance of IDL may be the result of an improved transfer of the surface components, phospholipid and cholesterol, to HDL where they can be eliminated by the HL reaction.

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IV

TREATMENT OF HYPERTHYROIDISM: EFFECTS ON HEPATIC LIPASE,
LIPOPROTEIN LIPASE, LCAT AND PLASMA LIPOPROTEINS

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ABSTRACT

The activities of hepatic lipase and of lipoprotein lipase, the elimination rate of exogenous triglyceride and the cholesterol esterification rate were determined and related to plasma lipoprotein concentrations in 16 patients before and after treatment for hyperthyroidism.

The activity of hepatic lipase was significantly higher (65%) before than after treatment, while the activity of lipoprotein lipase and the elimination rate of exogenous triglyceride remained unchanged. The endogenous cholesterol esterifying ability decreased after treatment, whereas no change occurred in the fractional cholesterol esterification rate measured with normal plasma as substrate. The concentrations of LDL-cholesterol and HDL-cholesterol increased significantly after treatment.

The decrease in hepatic lipase activities was correlated to the decrease in $S-T_3$ concentrations ($r = 0.77$, $\underline{P} < 0.001$) and to the increase in HDL cholesterol concentrations ($r = 0.51$, $\underline{P} < 0.05$). The activities of lipoprotein lipase were positively correlated to the concentrations of HDL-cholesterol both before ($r = 0.54$, $\underline{P} < 0.05$) and after ($r = 0.59$, $\underline{P} < 0.05$) treatment.

These results support the view that hepatic lipase and lipoprotein lipase are both important determinants of plasma HDL concentrations and strongly suggest that an increased hepatic lipase activity contributes to the lower plasma cholesterol concentrations, especially in the HDL fraction, in hyperthyroid patients.

INTRODUCTION

Hyperthyroidism is accompanied by decreased serum levels of LDL cholesterol and HDL cholesterol while changes in serum triglyceride concentrations (S-TG) are less prominent [1,3,17,18,29]. These changes in the lipoprotein pattern are essentially opposite to those found in severe forms of hypothyroidism [15,25,29] where decreased activities of lipoprotein lipase (LPL) and hepatic lipase (HL) seem to be of pathogenetic importance for the disturbances of the lipoprotein metabolism [2,12,15,28].

Results from earlier studies on total postheparin lipolytic activity in plasma from hyperthyroid patients are contradictory [4,11,18] and inconclusive regarding the separate activities of LPL and HL. Information on the individual enzyme activities in hyperthyroidism is scarce. One recent study on hyperthyroid patients before and after treatment demonstrated no significant changes in LPL activity or HL activities [2]. However, after tri-iodothyronine administration to healthy volunteers a rapid, selective rise in HL activity has been observed [10].

Information on lecitin: cholesterol acyl transferase (LCAT), another key enzyme in lipoprotein metabolism, in patients with thyroid dysfunction is limited. An increased fractional cholesterol esterification rate has been found in hyperthyroid patients but it is not clear whether this is due to increased LCAT activity or to changes in its substrate, leading to increased efficiency of the enzyme [13].

In the present study we have investigated patients with hyperthyroidism before and after treatment in order to further penetrate the influence of thyroid hyperfunction on the activities of LPL, HL and LCAT and to elucidate the role of these enzymes for the lipoprotein disturbances in this disease.

MATERIAL AND METHODS

Patients

Sixteen consecutive patients participated in the study.

Eleven were women, mean age 42.3 (range 21-60) years and five were men, mean age 39.6 (range 18-59) years. They were referred to the Department of Medicine because of clinical signs of hyperthyroidism, and the diagnosis was confirmed by triiodothyronine concentrations in serum ($S-T_3$) clearly above the upper reference limit (Table I). They had no signs of other diseases, and were not on treatment with drugs or diets at the time of the initial investigations. The project was approved by the local Ethical committee, and all subjects gave informed consent.

Ten patients received radioiodine therapy and six patients were treated with carbimazole (Neo Mercazole, Nicholas, U.K.). Of these six patients one was operated on with subtotal thyroidectomy.

The patients were reinvestigated when they had been euthyroid for at least three months. Then one of the radioiodine treated patients was on replacement therapy with l-thyroxine (Levaxin, Nyegaard, Norway), five patients were still on carbimazole, combined with l-thyroxine when needed, while the rest of the patients were without drugs.

Between the investigations the mean body weight increased from 56.8 (range 44-74) to 60.1 (range 47-70) kg in the women and from 68.0 (range 54-85) to 72.0 (range 53-89) kg in the male patients.

Analytical methods

Blood samples were drawn at 8 a.m. after the patients had been fasting for at least 12 h. Sampling for the determination of lipase activities and the intravenous fat tolerance test were performed on separate days.

All measurements of serum hormone levels were made by radio-ligand assays [23,24,26]. The saturation of thyroid hormone binding proteins was determined by the T_3 resin uptake test carried out according to Nosslin [22]. A free thyroxine index (FTI) was calculated as the product of $S-T_4$ and the value of the T_3 resin uptake test.

HDL cholesterol was determined in the clear supernatant obtained after precipitation of VLDL and LDL by $MgCl_2$ and

dextrane sulphate [7]. LDL cholesterol was calculated according to the formula, $\text{LDL cholesterol} = \text{P-cholesterol} - (\text{HDL cholesterol} + 0.45 \times \text{P-triglyceride})$ [8]. The lipoprotein patterns were always checked by agarose gel electrophoresis.

Plasma samples for the determination of lipase activities were drawn 15 min after the intravenous administration of 50 U heparin (Vitrum, Stockholm, Sweden) per kg body weight [20]. The activities of hepatic lipase and lipoprotein lipase were determined by specific methods using tri $[^3\text{H}]$ -oleoyl glycerol as substrate [20]. The elimination rate of exogenous triglyceride was determined by the intravenous fat tolerance test (IVFTT) as described by Carlsson & Rössner [5], using one ml of 10% Intralipid (Vitrum, Stockholm, Sweden) per kg body weight. Two measurements reflecting LCAT activity were used. The method of Stokke & Norum [27] measures the cholesterol esterification rate in the patient's plasma, while exogenous normal plasma is used as substrate in the measurement described by Glomset & Wright [9].

Statistical methods

The significance of differences was evaluated using Student's t-test. Correlations between different variables were analyzed by linear regression analysis.

Results

When reinvestigated, all patients had been relieved of their hyperthyroid symptoms, were clinically euthyroid, and had S-TSH and S-T₃ levels within the reference ranges (Table I).

Plasma cholesterol levels increased in all patients, on the average by 29%. The relative rise in LDL and HDL cholesterol concentrations were similar (Table I). The mean plasma triglyceride level, however, decreased significantly (Table I).

Individual values for the activities of HL and LPL and for the elimination rate of exogenous triglyceride are given in Fig 1. All but two patients showed a decrease in HL activity; the mean value $\pm$ SEM was 631.7 ± 54.4 mU/ml before and $379.7 \pm$

TABLE I

Variables reflecting thyroid function and lipoprotein concentrations in 16 patients before and after treatment for hyperthyroidism.

	S-T ₃ nmol/l	S-T ₄ nmol/l	FTI	P-cho1 mmol/l	LDL-cho1 mmol/l	HDL-cho1 mmol/l	P-TG mmol/l
Before	8.3 ± 0.8	267.4 ± 14.3	463.3 ± 36.6	4.04 ± 0.19	2.55 ± 0.18	0.99 ± 0.06	1.11 ± 0.11
After	2.0 ± 0.1 ^a	86.4 ± 5.8 ^a	78.4 ± 6.9 ^a	5.22 ± 0.26 ^a	3.55 ± 0.21 ^a	1.31 ± 0.10 ^b	0.81 ± 0.08 ^c
Reference range	0.9 - 3.2	57 - 154		3.2 - 8.2	1.7 - 4.4	0.8 - 1.6	0.4 - 2.1

Data are given as mean ± SEM.

a = $P < 0.001$ b = $P < 0.01$ c = $P < 0.05$ vs values before treatment.

38.1 mU/ml after therapy ($\underline{P} < 0.001$). Changes in LPL activity and IVFTT values were inconsistent and did not reach statistical significance. The mean LPL activity decreased from 119.0 ± 8.8 mU/ml to 107.9 ± 10.7 mU/ml while the mean elimination rate of exogenous triglyceride was 6.42 ± 0.60 %/min before and 7.54 ± 0.71 %/min after therapy.

The cholesterol esterification rate was measured in 12 patients (Fig 2). The endogenous cholesterol esterifying ability measured according to Stokke & Norum, decreased significantly from 7.23 ± 0.56 %/h to 4.70 ± 0.41 %/h ($\underline{P} < 0.005$). However, no significant difference was found in the fractional esterification rate as determined by the method of Glomset & Wright; the mean values were 2.86 ± 0.15 %/6h before and 2.76 ± 0.10 %/6h after therapy, respectively.

The S-T₃ concentrations were well correlated to the FTI values before treatment ($r = 0.89$, $\underline{P} < 0.001$). Of these two variables S-T₃ returned the best coefficients of correlation when related to enzyme activities and lipoprotein concentrations.

Before treatment the S-T₃ levels were correlated to the LDL cholesterol concentrations ($r = -0.65$, $\underline{P} < 0.01$). The S-T₃ levels were significantly correlated to the activities of HL before treatment ($r = 0.62$, $\underline{P} < 0.01$). Also, the decrease in S-T₃ levels was significantly correlated to the decrease in HL activities ($r = 0.77$, $\underline{P} < 0.001$; Fig 3). There were no significant correlations between the variables reflecting thyroid function and LPL activities.

The decrease in HL activity was significantly correlated to the increase in HDL cholesterol levels ($r = 0.51$, $\underline{P} < 0.05$) but not to that in LDL cholesterol. LPL activities and HDL cholesterol concentrations before and after treatment were significantly correlated ($r = 0.54$, $\underline{P} < 0.05$ and $r = 0.59$, $\underline{P} < 0.05$, respectively), but no correlation between the changes in these variables was observed.

The cholesterol esterifying ability, measured according to Stokke & Norum, was correlated to S-T₃ levels before treatment ($r = 0.65$, $\underline{P} < 0.05$). The changes in these variables after treatment were also correlated ($r = 0.53$, $\underline{P} < 0.05$).

No significant correlations were found between LCAT activi-

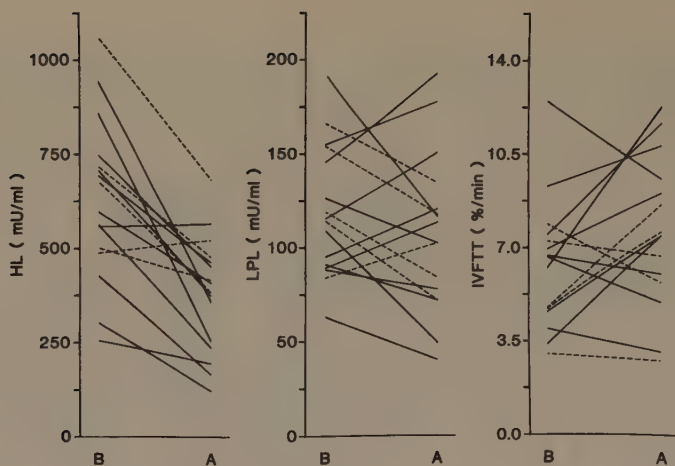


Fig 1. Activities of hepatic lipase (HL) and lipoprotein lipase (LPL) and the elimination rate of exogenous triglyceride (IVFTT) in sixteen patients before (B) and after (A) treatment for hyperthyroidism. Full lines indicate women, dotted lines men.

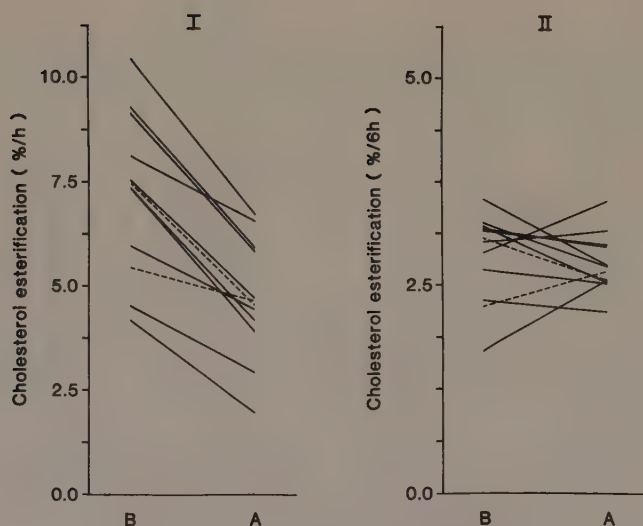


Fig 2. LCAT activities determined according to the methods of Stokke & Norum (I) and Glomset & Wright (II) in twelve patients before (B) and after (A) treatment for hyperthyroidism. Full lines indicate women, dotted lines men.

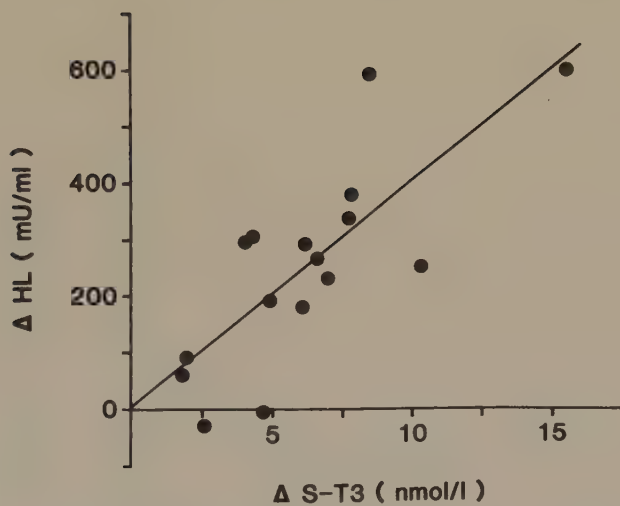


Fig 3. Relationship between changes in the activities of hepatic lipase (HL) and the serum levels of triiodothyronine (S-T3) during treatment of hyperthyroidism in sixteen patients. Regression equation: $y = 41.0 x + 1.03$; correlation coefficient = 0.77 ($P < 0.001$).

ties determined according to Glomset & Wright and thyroid hormone levels or lipoprotein concentrations.

DISCUSSION

The decreased level of plasma cholesterol, both in the LDL and in the HDL fraction, in our patients is in good agreement with earlier studies on hyperthyroid subjects as is the finding of only moderate changes in P-TG concentrations [1,3, 17,18]. In the present study we have investigated possible mechanisms behind these alterations by monitoring three enzyme activities which, in different ways, could all contribute to the changes in the lipoprotein profile. LPL catalyzes the degradation of the triglyceride-rich lipoproteins, VLDL and chylomicrons, to LDL; this process is also associated with transfer of lipoprotein surface components to HDL [21]. HL is considered to act as a phospholipase using HDL as its physiological substrate [19]. Both enzymes, therefore, may influence plasma HDL levels. LCAT is responsible for the esterification of cholesterol in plasma, and impaired LCAT activities lead to pronounced alterations in all the major lipoprotein classes [21].

It has recently been reported that the activity of HL decreased only insignificantly after treatment for hyperthyroidism [2]. In the present study the effect on HL activity was more pronounced, as our patients had significantly higher enzyme activities before than after treatment. This pattern is consistent with our recent study [10] demonstrating that T_3 administration to normal subjects rapidly leads to an increase in HL activity. The discrepancy with the study of Abrams et al [2] may be due to differences in experimental design (e g, the observation period before reinvestigation was longer in our study), but might also be related to the different lipase assays employed.

We have previously suggested that the influence of thyroid hormones on HL and LPL is exerted by different mechanisms: for example, the low HL activity in hypothyroidism responds much more rapidly to treatment than the subnormal LPL activity

[28], and experimental hyperthyroidism leads to a rapid rise in HL, but not LPL, activities [10]. The significant correlations between S-T₃ levels and HL activities before treatment as well as between the changes in these variables during treatment further supports our suggestion that the thyroid hormones exert a direct influence on HL [10,28]; evidently the elevated HL activities are maintained also during long term elevation of thyroid hormone levels. In contrast to HL, the activity of LPL remained essentially unchanged after treatment of our patients; this finding is in agreement with earlier studies [2,10]. The lack of changes in LPL activities during short term [10] as well as during long term influence of elevated levels of thyroid hormones indicates that LPL is not directly influenced by thyroid hormones.

Our results concerning LCAT activity confirm and extend previous observations [13]. The differential information obtained by the two methods indicates that the enzyme activity as such is not increased in hyperthyroid patients and suggests that substrate effects are of importance for the increased cholesterol esterifying ability in hyperthyroid patients. The selective increase observed by the assay of Stokke & Norum [27], may be related to the composition of the substrate, HDL, and/or the proportions of the HDL subclasses, HDL₂ and HDL₃. It has been reported [14] that hyperthyroid patients have a comparatively low HDL₂/HDL₃ ratio, which can be expected to favour the substrate preference of LCAT [16].

Data from clinical studies on the significance of LPL, HL and LCAT for disturbances in lipoprotein metabolism are often difficult to interpret since most pathophysiological conditions, e g hypothyroidism, are associated with changes in the activity of more than one of these enzymes. Hyperthyroidism, on the other hand, offers the possibility to study the effect on lipoprotein metabolism of a selective increase in the activity of HL. An interesting lipoprotein alteration in these patients with elevated HL activities was the low levels of HDL cholesterol, which normalized in parallel with the enzyme activity during treatment. The relationship between HL activity and HDL cholesterol concentrations is further corrobo-

rated by the significant correlation between the changes in HL activity and HDL cholesterol concentrations. In contrast, we found no relationships between HL activity and LDL cholesterol concentrations in these patients. These data support the view that HL plays an important role in the regulation of HDL concentrations and are consistent with the notion that the enzyme acts by facilitating removal of HDL phospholipid and cholesterol in the liver [19]. However, the positive correlations between individual values of LPL activities and HDL cholesterol concentrations are consistent with the view that LPL is also a determinant of HDL cholesterol concentrations, probably by promoting the input of surface components from VLDL and IDL to HDL [19].

The normal activity of LPL implies that the removal rate of VLDL triglyceride is normal in hyperthyroidism. This is consistent with the normal rate of elimination of exogenous triglyceride in our patients. The higher plasma triglyceride levels before therapy, decreasing significantly upon treatment, therefore do not seem to result from a triglyceride removal defect. Rather, the change in plasma triglyceride levels can be ascribed to alterations in VLDL production rate due to increased mobilization of fatty acid [18]. Also, the LPL dependent formation of LDL from the more triglyceride rich lipoproteins can be expected to proceed at a normal rate. The lower LDL concentrations in hyperthyroidism, therefore, seems to be due mainly to an enhanced elimination of LDL particles via the specific LDL receptor [6].

Acknowledgements

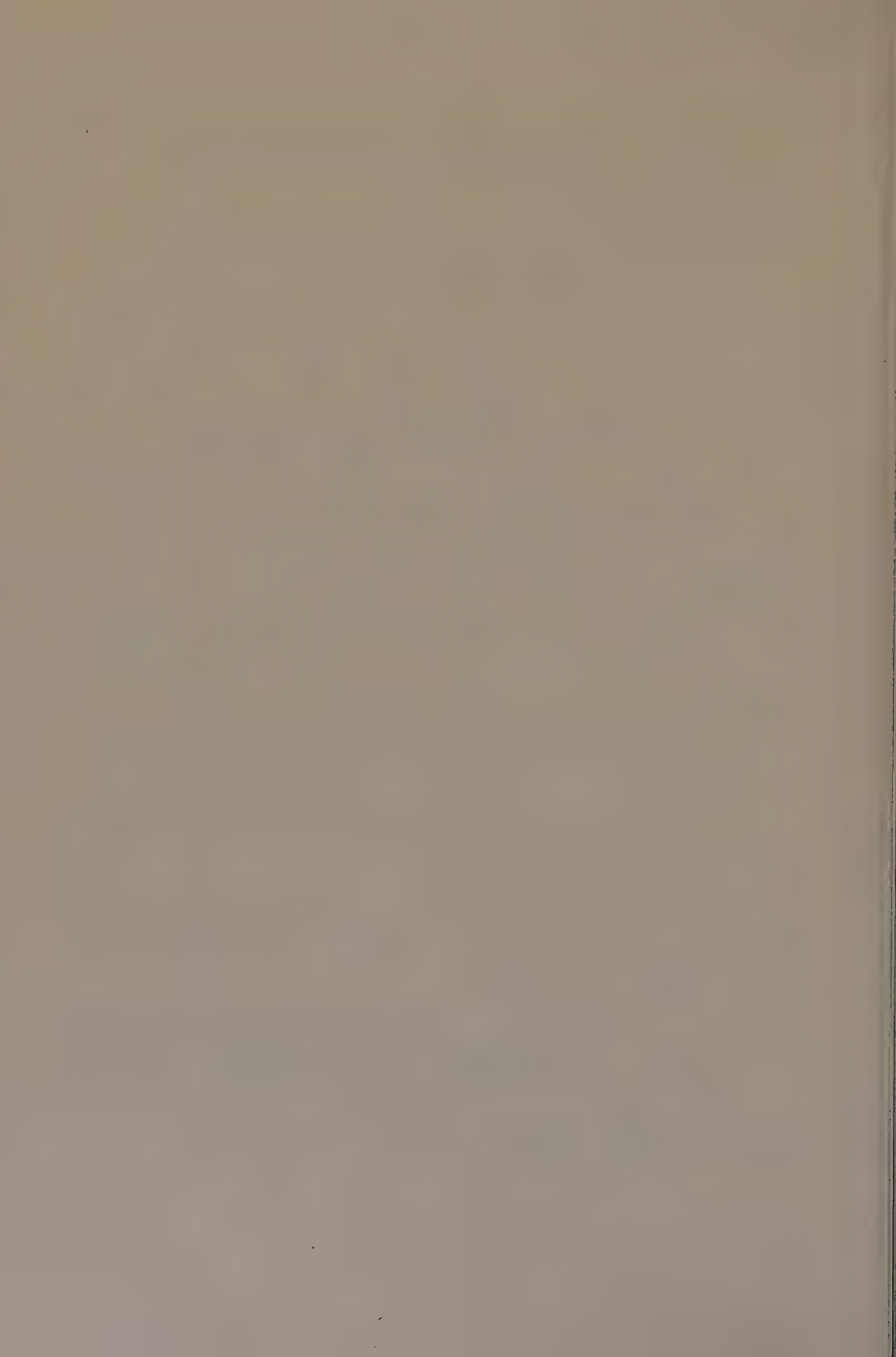
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Experimental Hyperthyroidism in Man: Effects on Plasma
Lipoproteins, Lipoprotein Lipase and Hepatic Lipase

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SUMMARY

We have studied the effects of triiodothyronine administration (20-40 ug three times daily over one week) in six healthy young men, on the activities of lipoprotein lipase and hepatic lipase and on plasma lipoprotein concentrations. Hepatic lipase activity in post-heparin plasma rose by $46 \pm 25\%$ ($p < 0.025$), whereas the activity of lipoprotein lipase did not change significantly. Plasma cholesterol concentrations decreased by about 20% ($p < 0.025$) whereas there was no change in plasma triglyceride levels. The fall in plasma cholesterol could be accounted for by a reduction of HDL cholesterol (-11%, $p < 0.025$) as well as LDL cholesterol (-27%, $p < 0.025$). The data emphasize the role of hepatic lipase in the lipoprotein alterations associated with thyroid dysfunction.

INTRODUCTION

Thyroid dysfunction is associated with alterations in the composition and concentrations of all major lipoprotein classes (Walton et al. 1965; Rössner and Rosenqvist 1974; Ballantyne et al. 1979; Scottolini et al. 1980; Agdeppa et al. 1979). The mechanisms responsible for the various dyslipoproteinemias are still incompletely understood. Besides the rate of production and elimination, the pattern of intravascular metabolism also influences the concentrations of plasma lipoproteins.

Recent studies indicate that alterations in the activities of lipoprotein lipase (LPL) and hepatic lipase (HL), two key enzymes in plasma lipoprotein metabolism, are important factors in the pathogenesis of dyslipoproteinemia seen in thyroid disease. Thus, LPL activity in adipose tissue (Pykälistö, Goldberg and Brunzell, 1976; Lithell et al. 1981), skeletal muscle (Lithell et al. 1981) and post-heparin plasma (Lithell et al. 1981; Valdemarsson, Hedner and Nilsson-Ehle 1982) is low in severely hypothyroid patients and increases on treatment. Mild thyroid hypofunction, however, does not seem to affect LPL activities (Lithell et al. 1981; Abrams, Grundy and Ginsberg 1981; Abrams and Grundy 1981). Studies on LPL activity in hyperthyroidism have yielded conflicting results (Abrams, Grundy and Ginsberg 1981; Abrams and Grundy 1981; Arons et al. 1972; Kirkeby 1968; Tulloch, Lewis and Fraser 1973; Nikkilä and Kekki 1972).

The effects of thyroid disease on HL activity have been less extensively studied. The enzyme activity is low in hypothyroidism (Valdemarsson, Hedner and Nilsson-Ehle 1982; Abrams, Grundy and Ginsberg 1981; Krauss, Levy and Fredrickson 1974), and substitution therapy rapidly restores HL activity to normal levels (Valdemarsson, Hedner and Nilsson-Ehle 1982; Abrams, Grundy and Ginsberg 1981). A recent study indicates that HL activities may be slightly elevated in hyperthyroidism (Abrams, Grundy and Ginsberg 1981).

In the present study we have investigated the time-course for the effects of experimental hyperthyroidism, induced by administration of triiodothyronine (T_3) to healthy subjects, on the activities of LPL and HL, and on plasma lipoprotein concentrations.

MATERIALS AND METHODS

Experimental design

The experiment was approved by the local Ethics Committee. Six men, aged 20 - 36 years, volunteered. They were all euthyroid and had no history or signs of endocrine or metabolic diseases. On Day 0, peroral administration of T_3 (Nyegaard A/A, Oslo, Norway), 20 ug three times daily, was started. On Day 2 the dose was doubled and the medication continued through day 6. Blood samples were obtained at 8 a.m. on days -5, -3, 1, 2, 4 and 7, the subjects fasting for at least 8 h. The initial values represent the mean of data obtained from the two samples collected prior to T_3 administration. Blood was drawn for the determination of T_3 , thyroxin (T_4), thyrotropin (TSH) and the T_3 uptake test, plasma cholesterol, HDL-cholesterol and triglycerides. Fifteen minutes after the intravenous injection of heparin (50 U/Kg bodyweight) blood was drawn for determination of LPL and HL activities in post-heparin plasma (Nilsson-Ehle and Ekman 1977). Separate control experiments demonstrated that the repeated heparin administration as such did not affect the lipoprotein concentration or lipase activities.

Analytical Methods

T_3 and TSH concentrations were determined by commercial radioimmunoassay kits (Skandinaviska Immunlaboratoriet AB, Malmö, Sweden). T_4 was determined by the method of Cheung and Slaunwhite (1976), using anti- T_4 - antiserum from Farnos Diagnostica, Turku, Finland.

The T_3 uptake test was performed according to Nosselin (1965).

Cholesterol and triglyceride were determined by enzymatic methods (Boehringer Mannheim GmbH, West Germany). HDL cholesterol was measured after precipitation of VLDL and LDL by dextran sulphate and $MgCl_2$ (Danielsson et al. 1978).

LDL-cholesterol was calculated by the formula: $LDL\text{-cholesterol} = \text{Total cholesterol} - (\text{HDL-cholesterol} + \text{Triglycerides} \times 0.45)$

(Modified to SI Units from Friedewald, Levy and Fredrickson 1972).

LPL and HL activities were determined by specific methods described earlier (Nilsson-Ehle and Ekman 1977), using dioleoyl phosphatidyl choline (final conc 0.067 mg/ml) instead of lyso-phosphatidyl choline as substrate emulsifier.

Statistics

The significance of differences was evaluated by Wilcoxon's rank order test for the paired case. Correlations between variables were evaluated by linear regression analysis.

RESULTS

On administration of T_3 five persons reported symptoms of hyperthyroidism. Serum T_3 levels increased to values above the upper reference limit in all subjects (Table I). The concentrations of TSH remained unchanged, whereas serum T_4 levels decreased continuously during the experiment (Table I). There was also a decrease in the T_3 uptake test.

HL activities increased rapidly in all subjects, roughly parallel to the rise in serum T_3 concentrations, to levels $46 \pm 25\%$ ($p < 0.025$) above the initial (Fig 1). Five of the 6 subjects reached enzyme activities above the upper reference limit. In contrast, the moderate rise in LPL activities (Fig 1) did not reach statistical significance.

The concentrations of plasma cholesterol, HDL-cholesterol, and LDL-cholesterol all gradually decreased (Fig II). The differences noted on day 4 and 7 reached statistical significance. The most pronounced change was recorded for LDL cholesterol (-27% , $p < 0.025$).

TABLE I

Laboratory tests reflecting thyroid function in six healthy subjects during triiodothyronine administration. Values are given as mean $\pm$ S.D. Asterisks indicate significances of differences compared to initial values. (* = $p < 0,05$; ** = $p < 0,025$). Reference values are those routinely used in our laboratory.

	Initial value (mean of two analyses in each person)	Day 1	Day 2	Day 4	Day 7	Reference range
T_3 (nmol/l)	$1,82 \pm 0,37$	$3,67 \pm 0,74$ ** *	$3,82 \pm 0,88$ ** *	$6,28 \pm 2,04$ ** *	$5,88 \pm 1,29$ * *	0,9 - 3,2
T_4 (nmol/l)	$85,2 \pm 11,6$	$88,2 \pm 11,9$	$67,0 \pm 14,9$ *	$62,2 \pm 15,2$ * *	$57,3 \pm 13,3$ * *	57 - 154
TSH mU/l	$2,6 \pm 1,5$	$2,1 \pm 0,9$	$1,8 \pm 0,9$	$2,5 \pm 0,6$	$2,0 \pm 0,5$	< 8
T_3 uptake (arbitrary units)	$1,12 \pm 0,14$	$0,99 \pm 0,09$ *	$0,94 \pm 0,08$ *	$1,00 \pm 0,07$ * *	$0,89 \pm 0,11$ * *	0,80 - 1,20

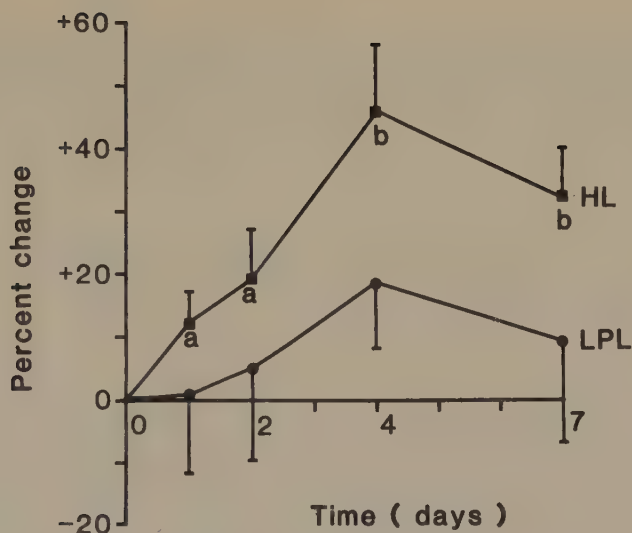


Fig 1. Time-course of changes in activities of hepatic lipase (HL) and lipoprotein lipase (LPL). The initial enzymatic activities (mean $\pm$ S.D.) were 506 ± 161 mU/ml plasma (HL) and 136 ± 38 mU/ml (LPL), respectively; one mU represents the release of one nmol fatty acid per minute. Bars represent SEM.

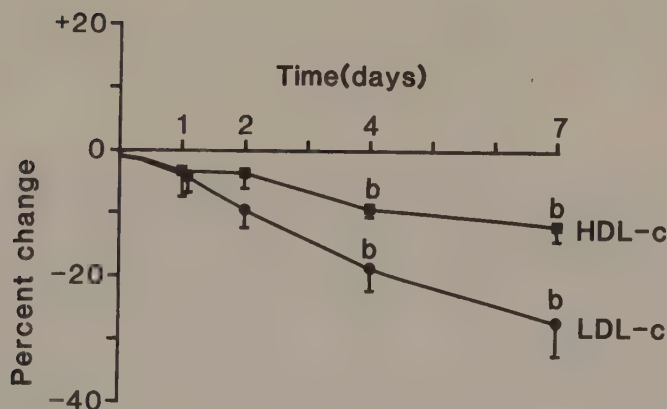


Fig 2. Relative changes in plasma HDL cholesterol and LDL cholesterol levels during triiodothyronine intake in healthy men. Initial concentrations were 1.12 ± 0.36 mmol/l and 3.40 ± 0.52 mmol/l (mean $\pm$ S.D.) respectively. Bars represent S.E.M.

The decrease in HDL-cholesterol averaged 12% ($p < 0.025$). The decrease in HDL cholesterol was equally pronounced in the subjects with initial values in the lower and upper reference range. No significant change was noted for the concentrations of plasma triglycerides.

There were no correlations between individual values for serum T_3 , HL activity or lipoprotein concentrations nor for the changes in these variables.

DISCUSSION AND CONCLUSIONS

In the present experiment we chose to induce experimental hyperthyroidism by administration of T_3 , which is the biologically more active thyroid hormone (Thorell and Larsson 1978; Chopra et al. 1978). The rise in T_3 levels was accompanied by a fall in T_4 concentrations, indicating suppression of the endogenous T_4 production. The limited sensitivity of the TSH radioimmunoassay (Thorell and Larsson 1978) probably explains why we could not detect a fall in TSH concentrations. The decreasing values of the T_3 uptake test are probably secondary to the decrease in T_4 levels, since the thyroid-hormone-binding proteins of plasma have a higher affinity for T_4 than for T_3 (Thorell and Larsson 1978).

Previous studies have shown that HL activity is low in hypothyroidism (Valdemarsson, Hedner and Nilsson-Ehle 1982; Abrams, Grundy and Ginsburg 1981; Krauss, Levy and Fredrickson 1972) and rises on substitution therapy (Valdemarsson, Hedner and Nilsson-Ehle 1982; Abrams, Grundy and Ginsberg 1981). This study extends these observations, demonstrating that HL activity in healthy subjects can be raised above the upper reference limit in experimental hyperthyroidism. The rise in enzyme activity was roughly parallel to the increase in T_3 concentrations. These data strongly suggest that T_3 plays a direct role in the physiological regulation of HL activity, which is consistent with earlier studies demonstrating a relationship between the increase in S- T_3 levels and HL activities recorded after substitution of

hypothyroid patients (Valdemarsson, Hedner and Nilsson-Ehle 1982). Thus it seems that alterations in HL activity accompany both hyper- and hypothyroidism (Abrams, Grundy and Ginsberg, 1981; Abrams and Grundy 1981).

The role of thyroid hormones in the regulation of LPL activity seems less clear-cut. Earlier studies indicate that this enzyme activity is low only in overt hypothyroidism and normalizes on treatment (Pykälistö, Goldberg and Brunzell 1976; Lithell et al. 1981; Abrams, Grundy and Ginsberg 1981; Abrams and Grundy 1981; Valdemarsson, Hedner and Nilsson-Ehle 1982). In subclinical thyroid hypofunction the enzyme activity seems essentially normal (Lithell et al. 1981). The present study demonstrates that the LPL activity responds poorly to an elevation of serum T_3 concentration, and despite clinical and laboratory signs of hyperthyroidism, our subjects showed no significant increase in LPL activity. Thus it seems that adequate concentrations of thyroid hormones are necessary for the maintenance of normal LPL activity. However, the iodothyronines seem to have a less prominent role in the short-term physiological regulation of LPL activities. This view is consistent with our suggestion (Valdemarsson, Hedner and Nilsson-Ehle 1982) that the effect of T_3 on LPL activity is mainly exerted through its positive effect on protein synthesis (Tata and Widnell 1966).

The observed decrease in HDL-cholesterol levels is consistent with studies in hyperthyroid patients, who generally have HDL-cholesterol levels in the lower reference range. (Agdeppa et al. 1979; Scottolini et al. 1980).

Agdeppa et al (1979) studied the effects of T_4 administration (0.3 mg daily for one month) on HDL-cholesterol concentrations in normal subjects, but could detect no change. The apparent discrepancy with our study may be explained by the different iodothyronines used. Their T_4 dose may have been too low to maintain sufficient levels of biologically active T_3 during their four weeks study (Thorell and Larsson 1978; Chopra et al. 1978).

The decrease in HDL-cholesterol, parallel with the selective increase HL activity, supports present views of HL as an enzyme involved in the degradation of HDL (Shirai, Barnhart and Jackson, 1981), probably acting as a phospholipase using HDL₂ as substrate. We did not quantitate the subfractions HDL₂ and HDL₃ separately in this study. However, extrapolation from another study (Brook et al. 1981) would indicate that the decrease was confined mainly to the HDL₂ subfraction.

LDL cholesterol concentrations decreased, as could be expected from earlier studies in hyperthyroid patients (Scottolini et al. 1980) as well as in normal subjects receiving an excess of T₄ (Agdeppa et al. 1979). The mechanisms may include both an increased number of LDL receptors (Chait, Bierman and Albers 1979) and an enhanced transfer of cholesterol and phospholipid to the HDL subfraction with subsequent rapid uptake in the liver via HL (Valdemarson, Hedner and Nilsson-Ehle 1982).

Acknowledgements

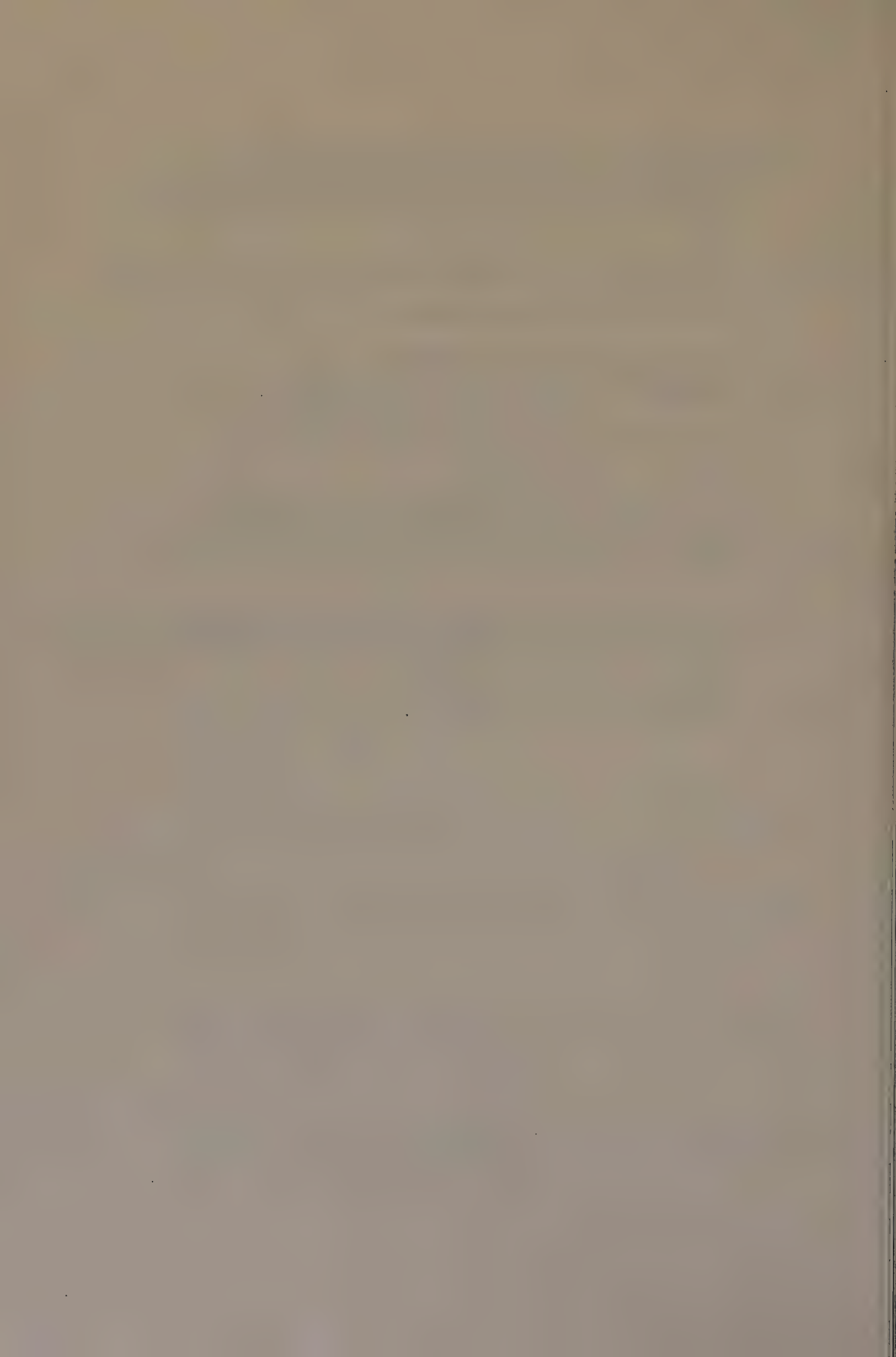
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DYSLIPOPROTEINEMIA IN HYPOTHYROIDISM OF PITUITARY ORIGIN:
EFFECTS OF L-THYROXINE SUBSTITUTION ON LIPOPROTEIN LIPASE,
HEPATIC LIPASE, AND ON PLASMA LIPOPROTEINS

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ABSTRACT

We have studied the effects of L-thyroxine substitution on lipoprotein concentrations, on the activities of lipoprotein lipase (LPL) and hepatic lipase (HL), and on the elimination rate of exogenous triglyceride in a homogenous group of patients with hypothyroidism of pituitary origin. All were deficient of sexual hormones but not of corticosteroids during the observation period.

Before treatment total plasma cholesterol, LDL cholesterol, and triglyceride levels were significantly higher than in a euthyroid control group but not as high as in patients with overt primary hypothyroidism. The activities of LPL and HL were also intermediate between those of euthyroid and overt primary hypothyroid subjects, and there was a significant reduction of the elimination rate of exogenous triglyceride. No changes were found for HDL cholesterol levels.

When the patients with secondary hypothyroidism were compared to patients with primary hypothyroidism, matched for thyroid function levels, age, sex, and weight, there were no differences with regard to plasma lipoprotein concentrations or postheparin lipase activities.

In 3 patients with secondary hypothyroidism the lipoprotein profiles were studied by zonal ultracentrifugation and found to agree well with changes observed in primary hypothyroidism.

L-thyroxine substitution produced a normalization of lipase activities and lipoprotein concentrations in patients with secondary hypothyroidism. We conclude that there are no fundamental differences in the disturbances of the lipoprotein metabolism in primary and secondary forms of hypothyroidism.

A prominent lipoprotein alteration in primary hypothyroidism is an increase in serum LDL cholesterol levels (Walton et al. 1965, Rössner & Rosenqvist 1974, Ballantyne et al. 1979). A close, inverse relationship has been found between serum triiodothyronine (S-T3) levels and LDL cholesterol concentrations (Valdemarsson et al. 1982 a). Decreased activities of hepatic lipase (HL) and lipoprotein lipase (LPL) seem to be of importance for the development of these alterations (Abrams et al. 1981, Valdemarsson et al. 1982 b).

It is frequently stated that the increase in serum cholesterol is often smaller or even absent in hypothyroidism secondary to pituitary failure in contrast to primary hypothyroidism (Bastenie et al. 1972, Utiger 1979, Dillon 1980, Ingbar & Woeber 1981, Christy 1982). It is not clear, however, whether this difference can be accounted for by different degrees of thyroid hypofunction or whether there are fundamental differences in lipid metabolism between patients with primary and secondary forms of thyroid hypofunction. The data on serum lipids and lipoproteins in pituitary insufficiency are based on studies in small and heterogeneous groups of patients with various multiple pituitary hormone defects (Summers et al. 1967, Sagel et al. 1979), and HL and LPL activities have not been studied in hypopituitarism.

In the present study we have monitored lipoprotein concentrations and lipase activities in a well defined group of patients with hypopituitarism but without hypocortisolism before and during l-thyroxine replacement therapy. The data were compared to those obtained in patients with various degrees of primary hypothyroidism, and in euthyroid subjects.

PATIENTS AND METHODS

Patients with hypothyroidism of pituitary origin

Five women (mean age 60.8 years, range 43 - 74 years) and six men (mean age 35.8 years, range 24 - 59 years) participated in the study.

Six patients had been treated for pituitary adenoma and one for craniopharyngeoma (surgery and/or radiotherapy). Two patients had small tumours in the sellar region considered not to need operation. Two patients had pituitary insufficiency without detectable tumours.

All the men had subnormal plasma levels of testosterone. One woman had amenorrhea with plasma levels of FSH and LH $<0.5 \mu\text{g/l}$. In four postmenopausal women basal gonadotropin levels were $<1.5 \mu\text{g/l}$. None of the patients received substitution with sexual hormones prior to or during the observation period.

The patient with craniopharyngeoma had a slight elevation of plasma prolactin that was left without treatment. The other patients had low or normal prolactin levels. All patients had low basal plasma levels of GH.

In seven patients replacement therapy with cortisone acetate, 37.5 mg daily, had been instituted prior to our investigations. In three patients with normal basal plasma levels of cortisol but defect cortisol response to insulin-induced hypoglycemia cortisone acetate treatment, 37.5 mg daily, was instituted parallel to 1-thyroxine.

Variables reflecting thyroid function are given in Table 1. The mean basal S-TSH level before 1-thyroxine substitution was $2.2 \pm 0.6 \text{ mU/l}$ (mean $\pm$ SEM) and the mean maximal increase after TRH ($200 \mu\text{g i.v.}$, sampling during 180 min) was $3.9 \pm 1.8 \text{ mU/l}$ (n.s.). Patients with central hypothyroidism may in some cases respond to TRH with an increase in S-TSH (Hershman 1980). The diagnosis of hypothyroidism of pituitary origin was therefore based on subnormal S-T3 and/or serum

thyroxine (S-T4) levels without increase in basal S-TSH concentrations in patients with known pituitary disease (Hershman 1980).

The patients were studied before substitution with 1-thyroxine, final dose 0.10 - 0.15 mg daily and when they had been euthyroid for at least 3 months. The mean body weight did not change significantly between the investigations (73.5 and 74.4 kg, respectively).

Patients with primary hypothyroidism and euthyroid subjects

Patients with overt primary hypothyroidism: 9 men and 15 women with elevated S-TSH and with S-T3 and S-T4 concentrations below the lower reference limits. Mean age was 48.2 years (range: 16 - 66) and mean body weight 74.0 kg.

Patients with subclinical primary hypothyroidism: 2 men and 10 women with elevated S-TSH levels, S-T4 low or normal, and S-T3 concentration within the reference range. Mean age was 49.9 years (range: 26 - 70) and mean body weight 64.8 kg.

Euthyroid subjects: 9 men and 8 women. Mean age was 34.7 years (range: 21 - 50) and mean body weight 75.5 kg.

From these groups 11 subjects were selected to match the patients with secondary hypothyroidism with regard to sex, age, body weight, and thyroid function level. Mean age in this group was 49.5 years (range: 21 - 70) and mean body weight 74.1 kg.

All patients studied gave informed consent. The study was approved by the Ethics Committee of the University of Lund.

Analytical methods

Thyroid hormones were determined by radioligand assays with reagents from MILAB (Malmö, Sweden). A free thyroxine index, FTI, was calculated from S-T4 and the degree of saturation of thyroid hormone binding proteins (Nosslin 1965). The

methods for determination of LPL and HL activities, plasma concentrations of TG, LDL and HDL cholesterol, and the intravenous fat tolerance test, IVFTT, have been given elsewhere (Valdemarsson et al. 1982 b). In three patients, plasma lipoproteins were separated in a Beckman Ti-14 zonal rotor by a two step procedure (Danielsson et al. 1978, Stubbe 1982), and the composition of the different lipoprotein classes determined as earlier described (Stubbe 1982).

RESULTS

Variables reflecting thyroid function, lipoprotein concentrations, and lipase activities are given in Table 1.

S-T3 and S-T4 levels in the patients with secondary hypothyroidism were intermediate between those recorded in the euthyroid subjects and those of the patients with primary overt hypothyroidism. The same pattern was found for total plasma cholesterol, LDL cholesterol, and for plasma triglyceride concentrations. No significant differences between the groups appeared for HDL cholesterol levels, demonstrating that the changes in plasma cholesterol were mainly confined to the LDL fraction.

The activities of HL and LPL in secondary hypothyroidism were lower than those in the euthyroid group; the LPL activities were almost equal to the low levels found in overt primary hypothyroidism. The impaired enzyme activity was paralleled by low IVFTT values which were almost the same in secondary and overt primary hypothyroidism, 2.74 ± 0.38 and 2.97 ± 0.25 %/min, respectively.

Fig 1 shows the relationship between S-T3 levels and LDL cholesterol concentrations in a group of subjects with primary hypothyroidism and healthy subjects. Data from the patients with secondary hypothyroidism were found to conform well with those of the patients with primary thyroid disease.

TABLE 1. Variables reflecting thyroid function, lipoprotein concentrations and activities of lipoprotein lipase (LPL) and hepatic lipase (HL) in patients with thyroid hypofunction of pituitary (secondary) and primary origin and in euthyroid subjects. Data are given as mean $\pm$ SEM.

	S-T3 nmol/l	S-T4 nmol/l	P-cho1 mmol/l	LDL-cho1 mmol/l	HDL-cho1 mmol/l	P-TG mmol/l	LPL mU/ml	HL mU/ml
Euthyroid subjects n=17	1.93 $\pm$ 0.09 ^a	90.7 $\pm$ 3.7 ^a	5.13 $\pm$ 0.18 ^b	3.46 $\pm$ 0.18 ^b	1.26 $\pm$ 0.08	0.90 $\pm$ 0.11 ^b	130.4 $\pm$ 11.2 ^b	349.9 $\pm$ 40.9
Secondary hypo- thyroidism. Before therapy n=11	1.12 $\pm$ 0.09	41.2 $\pm$ 3.6	7.53 $\pm$ 0.67	5.61 $\pm$ 0.62	1.20 $\pm$ 0.22	1.57 $\pm$ 0.20	82.5 $\pm$ 10.6	297.5 $\pm$ 42.6
Secondary hypo- thyroidism. After therapy n=11	2.11 $\pm$ 0.14 ^a	110.3 $\pm$ 9.8 ^a	5.32 $\pm$ 0.29 ^b	3.70 $\pm$ 0.28 ^b	1.03 $\pm$ 0.12	1.33 $\pm$ 0.15	106.5 $\pm$ 18.0	444.9 $\pm$ 39.3 ^a
Overt primary hypothyroidism n=24	0.46 $\pm$ 0.04 ^a (n=20)	23.3 $\pm$ 0.9 ^a	9.65 $\pm$ 0.53 ^c	7.38 $\pm$ 0.50 ^c	1.27 $\pm$ 0.09	1.73 $\pm$ 0.16 (n=22 ^{1/})	77.0 $\pm$ 7.8	199.0 $\pm$ 18.5 ^c
Matched primary hypothyroidism n=11	1.11 $\pm$ 0.13	50.2 $\pm$ 7.6	7.51 $\pm$ 0.53	5.60 $\pm$ 0.49	1.23 $\pm$ 0.12	1.49 $\pm$ 0.23	97.8 $\pm$ 12.0	277.9 $\pm$ 32.2
Reference ranges (mean $\pm$ 2 SD)	0.9-3.2	57-154	3.2-8.9	1.7-4.4	0.8-1.6	0.4-2.1	110-220	220-520

Significance of difference versus patients with secondary hypothyroidism before substitution with l-thyroxine is indicated by : a= P <0.001, b= P <0.01, c= P <0.05
1/ Two patients with severe hypertriglyceridemia excluded.

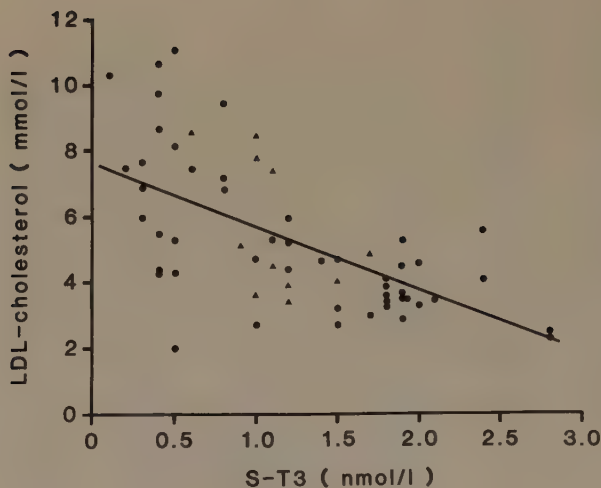


Fig 1. Relationships between S-T3 levels and LDL cholesterol concentrations in a group of subjects with thyroid function ranging from normal to low due to primary thyroid disease (dots, regression equation, $y = -1.98x + 7.66$; $r = -0.64$, $P < 0.001$) and in a group of patients with hypothyroidism of pituitary origin (triangles).

The mean S-T3 level in the matched group of patients with primary thyroid disease was almost identical with that found in the secondary hypothyroidism group. Also S-T4 concentrations, FTI (46.1 ± 9.3 and 34.2 ± 3.1 respectively) age, and body weight were closely similar. The lipoprotein concentrations were almost identical in the two groups. No significant differences were found for HL and LPL activities (Table 1).

L-thyroxine substitution of the patients with secondary hypothyroidism induced a significant decrease of total plasma and LDL cholesterol concentrations. HDL cholesterol and plasma triglyceride levels were also reduced, however not significantly. Both HL and LPL activities increased (Table 1). The elimination rate of exogenous triglyceride increased from 2.74 ± 0.38 and 4.21 ± 0.53 %/min ($P < 0.05$).

In general, the lipoprotein concentrations and lipase activities were normalized after treatment, i.e. were not significantly different from the values recorded in the euthyroid group. Only the plasma triglyceride levels remained elevated ($P < 0.05$ compared to the euthyroid group) after substitution.

In three patients with secondary hypothyroidism the lipoprotein profiles were studied by zonal ultracentrifugation before and after l-thyroxine substitution. The lipoprotein profile of one of these patients is shown in Fig. 2.

Besides an increase in the LDL concentration there was also an accumulation of intermediate density lipoproteins (IDL) and a relative increase of HDL₂ compared to HDL₃ particles before therapy. These changes were reduced after therapy. Data on the chemical composition of the IDL and HDL₂ fractions in these three patients are given in Table 2.

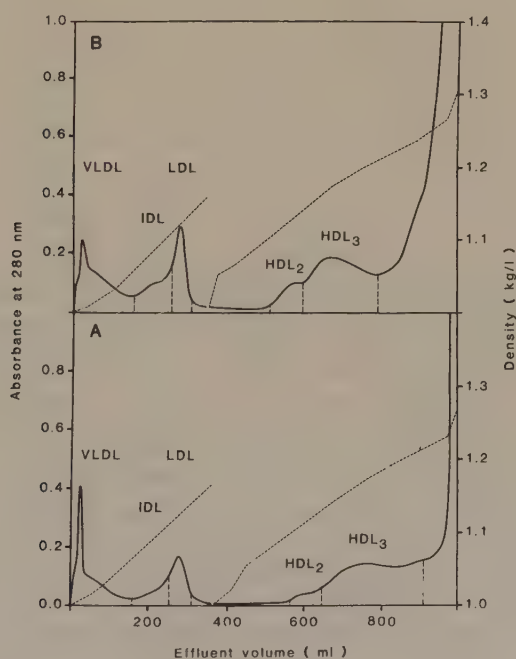


Fig 2. Lipoprotein profiles after separation of lipoproteins by zonal ultracentrifugation before (B) and after (A) l-thyroxine substitution in a male patient with hypothyroidism of pituitary origin (S-T3 = 1.2 nmol/l. S-T4 = 49 nmol/l, S-TSH = 1 mU/l). Full line indicates absorbance at 280 nm. Dotted lines indicate density gradient.

TABLE 2. Relative composition (% w/w) of representative subsamples of the IDL and HDL₂ fractions in three patients (1, 2, 3) with hypothyroidism of pituitary origin before (B) and after (A) l-thyroxine substitution. In one patient the IDL fraction could not be identified after therapy.

TG= triglyceride CE= cholesteryl ester
 FC= free cholesterol PL= phospholipid

Fraction	Patient	TG	CE	FC	PL	Protein
IDL	1 B	43.7	11.8	8.6	21.4	14.5
	A	12.8	36.3	8.6	22.7	19.6
	2 B	25.6	24.6	10.2	23.5	16.1
	A	15.6	32.8	7.2	20.4	24.0
	3 B	17.3	36.1	7.6	23.4	15.6
	A	-	-	-	-	-
HDL ₂	1 B	7.0	20.4	4.6	29.0	39.0
	A	10.3	19.4	2.9	22.9	44.5
	2 B	7.8	21.5	3.8	22.4	44.5
	A	16.5	11.2	6.7	22.8	42.8
	3 B	8.8	17.0	2.5	31.3	40.4
	A	9.9	16.8	3.7	27.1	42.7

DISCUSSION

Earlier reports on the effects on hyperlipidemia of hormone substitution, including thyroid hormones, in patients with hypopituitarism are inconclusive (Zöllner 1955, Tabatznik & Rabinowitz 1960, Jacobs et al. 1961). In a larger patient material Summers et al. (1967) found that hyperlipemia was not rare in hypopituitarism of various etiologies. Most of their patients with hyperlipemia had a low serum PBI. In 13 patients with multiple pituitary hormone deficiencies, including low S-T4 levels in 9, Sagel et al. (1979) found increased levels of triglyceride and cholesterol but a reduced HDL fraction, a pattern which is partly in agreement with that found in the present investigation.

Our patient material with multiple pituitary hormone defects was comparatively homogenous: none of the patients had cortisol deficiency, and all had S-T4 concentrations below the lower reference limit and deficient GH and gonadotropin functions at the start of the study. Qualitatively their lipoprotein alterations were similar to those seen in primary hypothyroidism with elevated LDL cholesterol and triglyceride levels. The presence of IDL and a relative increase in the HDL₂ subfraction is also in good agreement with findings in primary hypothyroidism (Lisch et al. 1982). In primary thyroid disease a relationship between the degree of thyroid dysfunction and lipoprotein alterations has been found (Valdemarsson et al. 1982 a) which makes it necessary to take into account the S-T3 levels for a quantitative comparison of lipoprotein changes in primary and secondary hypothyroidism. Our patients with pituitary dysfunction had a moderately impaired thyroid function, comparable to subclinical hypothyroidism. When compared to a matched group of patients with primary hypothyroidism, the lipoprotein changes in the patients with secondary hypothyroidism were also quantitatively almost identical. These patients also conformed well with the relationship between S-T3 levels and LDL cholesterol concentrations in patients with primary hypothyroidism. Thus,

the lipoprotein alterations in primary and secondary hypothyroidism, at least if there is no cortisol deficiency, seem identical and similarly related to the decrease in S-T3 levels.

The mechanism for the lipoprotein changes in primary hypothyroidism include impaired receptor mediated elimination of LDL particles (Thompson et al. 1981) and reduced activities of two of the key enzymes in the lipoprotein metabolism, LPL and HL (Abrams et al. 1981, Valdemarsson et al. 1982 b). Our patients with hypopituitarism showed reductions in LPL and HL activities similar to those found in the corresponding groups with primary hypothyroidism, and both enzyme activities normalized after l-thyroxine replacement. These findings demonstrate that there are no differences between the two forms of hypothyroidism with regard to these enzymes and indicate that disturbances in LPL and HL activities contribute to the dyslipoproteinemia in hypothyroidism of pituitary origin by similar mechanisms as has previously been suggested in primary hypothyroidism (Valdemarsson et al. 1982 a). The similarity between patients with primary and secondary hypothyroidism supports our earlier interpretation that the thyroid hormones as such influence the lipolytic enzymes (Valdemarsson et al. 1982 b) and that the elevation of S-TSH in primary hypothyroidism is of little importance for the low lipase activities in man. In the rat, in contrast, TSH has been reported to decrease adipose tissue LPL activity (Robinson & Wing 1970). After substitution therapy with l-thyroxine we found an almost complete normalization of the lipoprotein changes and the decreased lipase activities in the patients with secondary hypothyroidism. The persisting defects of sexual hormones and growth hormone, therefore, seem to be of little importance for the changes in lipoprotein metabolism. An isolated cortisol deficiency does not affect serum lipid levels (Summers et al. 1967). Thus the low S-T3 levels seem to fully account for the lipoprotein disturbances observed in hypopituitarism, and there does not seem to be any fundamental difference between lipoprotein changes found in primary and secondary hypothyroidism. The notion that

hypercholesterolemia in secondary hypothyroidism is less pronounced can probably be explained by the milder thyroid hormone deficiency usually seen in secondary hypothyroidism compared to the overt primary form of the disease (Hershman 1980).

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